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=> S (protease variant) (P) (unexpected substrate)
L1 0 (PROTEASE VARIANT) (P) (UNEXPECTED SUBSTRATE)

=> S (protease variant) (P) (unexpected cleavage)
L2 0 (PROTEASE VARIANT) (P) (UNEXPECTED CLEAVAGE)

=> S (protease variant) (P) (unexpected (3A) cleavage)
L3 0 (PROTEASE VARIANT) (P) (UNEXPECTED (3A) CLEAVAGE)

=> S (protease variant) (P) (unexpected (3A) substrate)
L4 0 (PROTEASE VARIANT) (P) (UNEXPECTED (3A) SUBSTRATE)

=> S (protease variant) (P) (unpredictable (3A) substrate)
L5 0 (PROTEASE VARIANT) (P) (UNPREDICTABLE (3A) SUBSTRATE)

=> S (protease variant) (P) (unpredictable (3A) cleavage)
L6 0 (PROTEASE VARIANT) (P) (UNPREDICTABLE (3A) CLEAVAGE)

=> S (protease or proteinase or peptidase) (3A) (variant or mutant or mutated or mutation or mutating or mutagenesis or substitution or substitute or substituted or substituting or replace or replaced or replacing or replacement or exchange or exchanged or exchanging)
L7 8630 (PROTEASE OR PROTEINASE OR PEPTIDASE) (3A) (VARIANT OR MUTANT OR MUTATED OR MUTATION OR MUTATING OR MUTAGENESIS OR SUBSTITUTION OR SUBSTITUTE OR SUBSTITUTED OR SUBSTITUTING OR REPLACE OR REPLACED OR REPLACING OR REPLACEMENT OR EXCHANGE OR EXCHANGED OR EXCHANGING)

=> S (protease or proteinase or peptidase) (4A) (unexpected or unexpectedly or unpredictable (2A) cleavage or cleaved or cleaving or cleaves)
L8 12942 (PROTEASE OR PROTEINASE OR PEPTIDASE) (4A) (UNEXPECTED OR UNEXPECTEDLY OR UNPREDICTABLE (2A) CLEAVAGE OR CLEAVED OR CLEAVING OR CLEAVES)

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=> s l7 and l8
L9          228 L7 AND L8

=> s l7 (P) l8
L10         161 L7 (P) L8

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=> d l11 1-51 bib ab

L11  ANSWER 1 OF 51     MEDLINE on STN                  DUPLICATE 1
AN   2009671757      IN-PROCESS
DN   PubMed ID: 19556225
TI   Insights into the enzyme-substrate interaction in the norovirus 3C-like
     protease.
AU   Someya Yuichi; Takeda Naokazu
CS   Department of Virology II, National Institute of Infectious Diseases,
     4-7-1 Gakuen, Musashi-Murayama, Tokyo 208-0011, Japan.. someya@nih.go.jp
SO   Journal of biochemistry, (2009 Oct) Vol. 146, No. 4, pp. 509-21.
     Electronic Publication: 2009-06-24.
     Journal code: 0376600. E-ISSN: 1756-2651. L-ISSN: 0021-924X.
CY   England: United Kingdom
DT   Journal; Article; (JOURNAL ARTICLE)
     (RESEARCH SUPPORT, NON-U.S. GOV'T)
LA   English
FS   NONMEDLINE; IN-PROCESS; NONINDEXED; Priority Journals
ED   Entered STN: 8 Oct 2009
     Last Updated on STN: 16 Dec 2009
AB   The Glu54 residue of the norovirus 3C-like protease was implicated in
     proteolysis as a third-member carboxylate of the catalytic triad.  The
     E54L mutant protease cleaved the sequence
     (133)LSFE/AP between the 3B and 3C regions of norovirus polyprotein, but
     did not cleave the sequence (198)ATSE/GK between the 3A and 3B.  The 3BC
     junction mutation (3B-L133A or 3B-F135S) hampered the cleavage by the E54L
     protease, whereas the 3AB junction mutation (3A-A198L, S200F) allowed the
     E54L protease to digest.  These results indicate that the E54L
     mutant protease is a substrate-specificity mutant and
     requires large hydrophobic amino acid residues at both P4 and P2 positions
     of the substrate.  It was notable that the 3A-S200F P2 position mutation
     caused tight interaction between the wild-type protease and the C-terminus
     of the 3A protein, hence a decreased release rate of the product from the
     enzyme.  This tight binding was dependent on the hydrophobicity of amino
     acid residues introduced at position 200 of the 3A region and was affected
     by the mutation in the bII-cII loop of the protease or the
     mutation of position 198 of 3A corresponding to the P4 position of
     the substrate.  These results suggest that the protease and the substrate
     sense each other in the process of the proteolysis, being supported by
     crystal structures.

L11  ANSWER 2 OF 51     MEDLINE on STN                  DUPLICATE 2
AN   2008333385      MEDLINE
DN   PubMed ID: 18400852
TI   Poliovirus 2A(Pro) increases viral mRNA and polysome stability
```

coordinately in time with cleavage of eIF4G.
AU Kempf Brian J; Barton David J
CS Department of Microbiology, University of Colorado Denver, School of Medicine, 12800 East 19th Ave., Aurora, CO 80045, USA.
NC AI42189 (United States NIAID NIH HHS)
T32 AI07537 (United States NIAID NIH HHS)
SO Journal of virology, (2008 Jun) Vol. 82, No. 12, pp. 5847-59. Electronic Publication: 2008-04-09.
Journal code: 0113724. E-ISSN: 1098-5514.
Report No.: NLM-PMC2395153.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, N.I.H., EXTRAMURAL)
LA English
FS Priority Journals
EM 200808
ED Entered STN: 24 May 2008
Last Updated on STN: 13 Aug 2008
Entered Medline: 12 Aug 2008
AB Poliovirus (PV) 2A protease (2A(Pro)) cleaves eukaryotic initiation factors 4GI and 4GII (eIF4GI and eIF4GII) within virus-infected cells, effectively halting cap-dependent mRNA translation. PV mRNA, which does not possess a 5' cap, is translated via cap-independent mechanisms within viral protease-modified messenger ribonucleoprotein (mRNP) complexes. In this study, we determined that 2A(Pro) activity was required for viral polysome formation and stability. 2A(Pro) cleaved eIF4GI and eIF4GII as PV polysomes assembled. A 2A(Cys109Ser) (2A(Pro) with a Cys109Ser mutation) protease active site mutation that prevented cleavage of eIF4G coordinately inhibited the de novo formation of viral polysomes, the stability of viral polysomes, and the stability of PV mRNA within polysomes. 2A(Cys109Ser)-associated defects in PV mRNA and polysome stability correlated with defects in PV mRNA translation. 3C(Pro) activity was not required for viral polysome formation or stability. 2A(Pro)-mediated cleavage of eIF4G along with poly(rC) binding protein binding to the 5' terminus of uncapped PV mRNA appear to be concerted mechanisms that allow PV mRNA to form mRNP complexes that evade cellular mRNA degradation machinery.

L11 ANSWER 3 OF 51 MEDLINE on STN DUPLICATE 3
AN 2008615010 MEDLINE
DN PubMed ID: 18710212
TI Automated molecular simulation based binding affinity calculator for ligand-bound HIV-1 proteases.
AU Sadiq S Kashif; Wright David; Watson Simon J; Zasada Stefan J; Stoica Ileana; Coveney Peter V
CS Centre for Computational Science, Department of Chemistry, University College London, London, WC1H 0AJ, UK.
SO Journal of chemical information and modeling, (2008 Sep) Vol. 48, No. 9, pp. 1909-19. Electronic Publication: 2008-08-19.
Journal code: 101230060. ISSN: 1549-9596.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
(RESEARCH SUPPORT, U.S. GOV'T, NON-P.H.S.)
LA English
FS Priority Journals
EM 200811
ED Entered STN: 23 Sep 2008
Last Updated on STN: 18 Nov 2008
Entered Medline: 17 Nov 2008

AB The successful application of high throughput molecular simulations to determine biochemical properties would be of great importance to the biomedical community if such simulations could be turned around in a clinically relevant timescale. An important example is the determination of antiretroviral inhibitor efficacy against varying strains of HIV through calculation of drug-protein binding affinities. We describe the Binding Affinity Calculator (BAC), a tool for the automated calculation of HIV-1 protease-ligand binding affinities. The tool employs fully atomistic molecular simulations alongside the well established molecular mechanics Poisson-Boltzmann solvent accessible surface area (MMPBSA) free energy methodology to enable the calculation of the binding free energy of several ligand-protease complexes, including all nine FDA approved inhibitors of HIV-1 protease and seven of the natural substrates cleaved by the protease. This enables the efficacy of these inhibitors to be ranked across several mutant strains of the protease relative to the wildtype. BAC is a tool that utilizes the power provided by a computational grid to automate all of the stages required to compute free energies of binding: model preparation, equilibration, simulation, postprocessing, and data-marshaling around the generally widely distributed compute resources utilized. Such automation enables the molecular dynamics methodology to be used in a high throughput manner not achievable by manual methods. This paper describes the architecture and workflow management of BAC and the function of each of its components. Given adequate compute resources, BAC can yield quantitative information regarding drug resistance at the molecular level within 96 h. Such a timescale is of direct clinical relevance and can assist in decision support for the assessment of patient-specific optimal drug treatment and the subsequent response to therapy for any given genotype.

L11 ANSWER 4 OF 51 CAPLUS COPYRIGHT 2009 ACS on STN
AN 2008:1535474 CAPLUS
DN 150:138397
TI The DEG15 serine protease cleaves peroxisomal targeting signal 2-containing proteins in Arabidopsis
AU Schuhmann, Holger; Huesgen, Pitter F.; Gietl, Christine; Adamska, Iwona
CS Department of Physiology and Plant Biochemistry, University of Konstanz, Konstanz, DE-78457, Germany
SO Plant Physiology (2008), 148(4), 1847-1856
CODEN: PLPHAY; ISSN: 0032-0889
PB American Society of Plant Biologists
DT Journal
LA English
AB Two distinct peroxisomal targeting signals (PTSs), the C-terminal PTS1 and the N-terminal PTS2, are defined. Processing of the PTS2 on protein import is conserved in higher eukaryotes. Recently, candidates for the responsible processing protease were identified from plants (DEG15) and mammals (TYSND1). We demonstrate that plants lacking DEG15 show an expressed phenotype potentially linked to reduced β-oxidation, indicating the impact of protein processing on peroxisomal functions in higher eukaryotes. Mutational anal. of Arabidopsis (Arabidopsis thaliana) DEG15 revealed that conserved histidine, aspartic acid, and serine residues are essential for the proteolytic activity of this enzyme in vitro. This indicates that DEG15 and related enzymes are trypsin-like serine endopeptidases. Deletion of a plant-specific stretch present in the protease domain diminished, but did not abolish, the proteolytic activity of DEG15 against the PTS2-containing glyoxysomal malate dehydrogenase. Fluorescence microscopy showed that a DEG15-green fluorescent protein fusion construct is targeted to peroxisomes in planta. In vivo studies with isolated homozygous deg15 knockout mutants and complemented mutant lines suggest that this enzyme mediates general

processing of PTS2-containing proteins.
OSC.G 1 THERE ARE 1 CAPLUS RECORDS THAT CITE THIS RECORD (1 CITINGS)
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L11 ANSWER 5 OF 51 MEDLINE on STN DUPLICATE 4
AN 2008676424 MEDLINE
DN PubMed ID: 18674574
TI Sapovirus-like particles derived from polyprotein.
AU Hansman Grant S; Oka Tomoichiro; Takeda Naokazu
CS Department of Virology II, National Institute of Infectious Diseases, Japan.. g@nih.go.jp
SO Virus research, (2008 Nov) Vol. 137, No. 2, pp. 261-5. Electronic Publication: 2008-08-15.
Journal code: 8410979. ISSN: 0168-1702.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LA English
FS Priority Journals
EM 200901
ED Entered STN: 23 Oct 2008
Last Updated on STN: 7 Jan 2009
Entered Medline: 6 Jan 2009
AB We expressed full-length sapovirus genome constructs in insect cells and analyzed their products. The capsid protein was cleaved from the ORF1 polyprotein from a native-like genome construct and two full-length genome constructs with mutations in an active polymerase motif, whereas the capsid protein was not cleaved from a full-length genome construct with a mutation in an active protease motif. Our results
showed that the sapovirus protease-polymerase precursor protein cleaved the capsid protein from the polyprotein at the putative conserved capsid start. Importantly, the cleaved capsid protein formed empty virus-like particles that were morphologically and antigenically similar to native sapovirus.

L11 ANSWER 6 OF 51 EMBASE COPYRIGHT (c) 2009 Elsevier B.V. All rights reserved on STN DUPLICATE 5
AN 2009033996 EMBASE
TI Design of mutation-resistant HIV protease inhibitors with the substrate envelope hypothesis.
AU Chellappan, S.; Reddy, G.S.K.K.; Ali, A.
SO Chemtracts, (March 2008) Vol. 21, No. 3, pp. 103-104.
ISSN: 1431-9268 CODEN: CHEMFW
PB Data Trace Publishing Company, 110 West Road, Ste. 227, Towson, Maryland, MD 21204-2316, United States.
CY United States
DT Journal; Article
FS 004 Microbiology: Bacteriology, Mycology, Parasitology and Virology
030 Clinical and Experimental Pharmacology
037 Drug Literature Index
LA English
SL English
ED Entered STN: 6 Feb 2009
Last Updated on STN: 6 Feb 2009
AB The introduction of human immunodeficiency virus protease (HIVP) inhibitors as therapeutic agents against HIV has considerably improved survival rate of acquired immunodeficiency syndrome (AIDS) patients. However, treatment using these inhibitors is hampered by the emergence of resistance mutations within the viral protease that decrease inhibitor affinity. Thus, to retain efficacy as anti-HIV drugs, the inhibitors must

retain the ability to bind to HIVP despite the presence of mutations in the enzyme. This article reports on the use of the so-called "substrate envelope hypothesis" as an approach to search for inhibitors of HIVP that remain effective despite the presence of resistance mutations. The natural substrates of the HIVP (i.e., sites in the Gag-Pol polyprotein that are cleaved by the protease) all occupy essentially the same area of the substrate binding site despite having differing amino acid sequences. Importantly, mutations that give rise to inhibitor resistance mostly lie outside this substrate envelope, presumably because mutations within the envelope would compromise the essential cleavage of the viral polyprotein. Thus, an inhibitor that fits within the envelope should be immune to resistance mutations. The investigators designed a combinatorial chemical library using computational methods and based on the hydroxyethylaminosulfonamide scaffold (Fig. 1) as a blueprint of the HIVP inhibitors. Additionally, the authors used combined docking and substrate envelope scores to identify and synthesize two compounds that were tested against wild-type and mutant HIV-1 protease for inhibitory activities (Fig. 2). The two compounds were determined to have considerable affinity against HIVP mutants, although lower than that of Amprenavir, which is better fitted to the conformation of the substrate envelope. .COPYRGT. 2008 Data Trace Publishing Company.

L11 ANSWER 7 OF 51 MEDLINE on STN DUPLICATE 6
AN 2007239056 MEDLINE
DN PubMed ID: 17337448
TI Functional characterization of cis and trans activity of the Flavivirus NS2B-NS3 protease.
AU Bera Aloke K; Kuhn Richard J; Smith Janet L
CS Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907, USA.
NC P01 AI-055672 (United States NIAID NIH HHS)
SO The Journal of biological chemistry, (2007 Apr 27) Vol. 282, No. 17, pp. 12883-92. Electronic Publication: 2007-03-02.
Journal code: 2985121R. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, N.I.H., EXTRAMURAL)
LA English
FS Priority Journals
EM 200706
ED Entered STN: 24 Apr 2007
Last Updated on STN: 15 Jun 2007
Entered Medline: 14 Jun 2007
AB Flaviviruses are serious human pathogens for which treatments are generally lacking. The proteolytic maturation of the 375-kDa viral polyprotein is one target for antiviral development. The flavivirus serine protease consists of the N-terminal domain of the multifunctional nonstructural protein 3 (NS3) and an essential 40-residue cofactor (NS2B(40)) within viral protein NS2B. The NS2B-NS3 protease is responsible for all cytoplasmic cleavage events in viral polyprotein maturation. This study describes the first biochemical characterization of flavivirus protease activity using full-length NS3. Recombinant proteases were created by fusion of West Nile virus (WNV) NS2B(40) to full-length WNV NS3. The protease catalyzed two autolytic cleavages. The NS2B/NS3 junction was cleaved before protein purification. A second site at Arg(459) decreasing Gly(460) within the C-terminal helicase region of NS3 was cleaved more slowly. Autolytic cleavage reactions also occurred in NS2B-NS3 recombinant proteins from yellow fever virus, dengue virus types 2 and 4, and Japanese encephalitis virus. Cis and trans cleavages were distinguished using a noncleavable WNV protease

variant and two types of substrates as follows: an inactive variant of recombinant WNV NS2B-NS3, and cyan and yellow fluorescent proteins fused by a dodecamer peptide encompassing a natural cleavage site. With these materials, the autolytic cleavages were found to be intramolecular only. Autolytic cleavage of the helicase site was insensitive to protein dilution, confirming that autolysis is intramolecular. Formation of an active protease was found to require neither cleavage of NS2B from NS3 nor a free NS3 N terminus. Evidence was also obtained for product inhibition of the protease by the cleaved C terminus of NS2B.

L11 ANSWER 8 OF 51 MEDLINE on STN DUPLICATE 7
AN 2007379218 MEDLINE
DN PubMed ID: 17475644
TI Identification of a Ca2+-binding domain in the rubella virus nonstructural protease.
AU Zhou Yubin; Tzeng Wen-Pin; Yang Wei; Zhou Yumei; Ye Yiming; Lee Hsiau-wei; Frey Teryl K; Yang Jenny
CS Department of Chemistry, Georgia State University, 50 Decatur St., Atlanta, GA 30303, USA.
NC GM070555 (United States NIGMS NIH HHS)
GM62999 (United States NIGMS NIH HHS)
R01 AI21389 (United States NIAID NIH HHS)
SO Journal of virology, (2007 Jul) Vol. 81, No. 14, pp. 7517-28. Electronic Publication: 2007-05-02.
Journal code: 0113724. ISSN: 0022-538X.
Report No.: NLM-PMC1933374.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, N.I.H., EXTRAMURAL)
(RESEARCH SUPPORT, U.S. GOV'T, NON-P.H.S.)
LA English
FS Priority Journals
EM 200709
ED Entered STN: 29 Jun 2007
Last Updated on STN: 15 Sep 2007
Entered Medline: 14 Sep 2007
AB The rubella virus (RUB) nonstructural protein (NS) open reading frame (ORF) encodes a polypeptide precursor that is proteolytically self cleaved into two replicase components involved in viral RNA replication. A putative EF-hand Ca(2+)-binding motif that was conserved across different genotypes of RUB was predicted within the nonstructural protease that cleaves the precursor by using bioinformatics tools. To probe the metal-binding properties of this motif, we used an established grafting approach and engineered the 12-residue Ca(2+)-coordinating loop into a non-Ca(2+)-binding scaffold protein, CD2. The grafted EF-loop bound to Ca(2+) and its trivalent analogs Tb(3+) and La(3+) with K(d)s of 214, 47, and 14 microM, respectively. Mutations (D1210A and D1217A) of two of the potential Ca(2+)-coordinating ligands in the EF-loop led to the elimination of Tb(3+) binding. Inductive coupled plasma mass spectrometry was used to confirm the presence of Ca(2+) ([Ca(2+)]/[protein] = 0.7 +/- 0.2) in an NS protease minimal metal-binding domain, RUBCa, that spans the EF-hand motif. Conformational studies on RUBCa revealed that Ca(2+) binding induced local conformational changes and increased thermal stability (Delta T(m) = 4.1 degrees C). The infectivity of an RUB infectious cDNA clone containing the mutations D1210A/D1217A was decreased by approximately 20-fold in comparison to the wild-type (wt) clone, and these mutations rapidly reverted to the wt sequence. The NS protease containing these mutations was less efficient at precursor cleavage than the wt NS protease at 35 degrees C, and the mutant NS protease was temperature sensitive at 39 degrees C, confirming

that the Ca(2+)-binding loop played a structural role in the NS protease and was specifically required for optimal stability under physiological conditions.

L11 ANSWER 9 OF 51 MEDLINE on STN DUPLICATE 8
AN 2006671183 MEDLINE
DN PubMed ID: 17109345
TI A complete mutational fitness map of the hepatitis C virus nonstructural 3 protease: relation to recognition by cytotoxic T lymphocytes.
AU Soderholm Jonas; Sallberg Matti
CS Division of Clinical Virology, Karolinska Institutet at Karolinska University Hospital Huddinge, S-141 86, Stockholm, Sweden.
SO The Journal of infectious diseases, (2006 Dec 15) Vol. 194, No. 12, pp. 1724-8. Electronic Publication: 2006-11-03.
Journal code: 0413675. ISSN: 0022-1899.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 200701
ED Entered STN: 21 Nov 2006
Last Updated on STN: 18 Jan 2007
Entered Medline: 17 Jan 2007
AB The hepatitis C virus nonstructural (NS) 3/4A protease sequence is highly conserved for reasons not fully understood. We determined the protease activity in 181 NS3/4A gene products in which each protease residue was replaced by alanine or glycine. Unexpectedly, most (87%) protease residues could be replaced and protease activity would be retained. Using these data, we were able to identify a human leukocyte antigen A2-restricted epitope in which substitutions at 5 of 9 residues destroyed the protease. The NS3 protease shows an unexpectedly high plasticity, and it is therefore important to identify target sequences in which the appearance of mutations is restricted by viral fitness.

L11 ANSWER 10 OF 51 MEDLINE on STN DUPLICATE 9
AN 2006574977 MEDLINE
DN PubMed ID: 17003922
TI In-vitro and in-vivo consequences of mutations in the von Willebrand factor cleaving protease ADAMTS13 in thrombotic thrombocytopenic purpura.
AU Donadelli Roberta; Banterla Federica; Galbusera Miriam; Capoferri Cristina; Bucchioni Sara; Gastoldi Sara; Nosari Silvia; Monteferrante Giuseppe; Ruggeri Zaverio M; Bresin Elena; Scheiflinger Friedrich; Rossi Edoardo; Martinez Constantino; Coppo Rosanna; Remuzzi Giuseppe; Noris Marina
CS Mario Negri Institute for Pharmacological Research, Clinical Research Center for Rare Diseases, Villa Camozzi, Via Camozzi, 3, 24020 Ranica, Bergamo, Italy. (International Registry of Recurrent and Familial HUS/TTP).
SO Thrombosis and haemostasis, (2006 Oct) Vol. 96, No. 4, pp. 454-64.
Journal code: 7608063. ISSN: 0340-6245.
CY Germany: Germany, Federal Republic of
DT Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LA English
FS Priority Journals
EM 200611
ED Entered STN: 28 Sep 2006
Last Updated on STN: 14 Nov 2006

Entered Medline: 13 Nov 2006

AB Thrombotic thrombocytopenic purpura (TTP) is a disease characterized by microvascular thrombosis, often associated with deficiency of the vonWillebrand factor (VWF) cleaving protease ADAMTS13. We investigated the spectrum of ADAMTS13 gene mutations in patients with TTP and congenital ADAMTS13 deficiency to establish the consequences on ADAMTS13 processing and activity. We describe five missense (V88M, G1239V, R1060W, R1123C and R1219W), 1 nonsense (W1016Stop) and 1 insertion (82_83insT) mutations. In two patients no mutation was identified despite undetectable protease activity. Expression in HEK293 mammalian cells (V88M, G1239V, R1123C and R1219W) documented that three missense mutants were not secreted, whereas theV88M was secreted at low levels and with reduced activity. We also provide evidence that impaired secretion of ADAMTS13 mutants observed in vitro translates into severely reduced ADAMTS13 antigen levels in patients in vivo. To evaluate whether the small amounts of mutant protease present in the circulation of patients had VWF cleaving activity, WT and mutant rADAMTS13 were stably expressed in Drosophila S2 cells under the influence of the Drosophila BiP protein signal sequence, which allows protein secretion. Drosophila expression system showed a 40-60% protease activity in the mutants. Several single nucleotide polymorphisms (SNPs) within exons and intron boundaries were found in patients, suggesting that the interplay of SNPs could at least in part account for ADAMTS13 functional abnormalities in patients without mutations. In conclusion, defective secretion and impaired activity of the mutants concur to determine an almost complete deficiency of ADAMTS13 activity in patients with a homozygous or two heterozygous ADAMTS13 mutations.

L11 ANSWER 11 OF 51 SCISEARCH COPYRIGHT (c) 2009 The Thomson Corporation on STN

AN 2006:688128 SCISEARCH

GA The Genuine Article (R) Number: 060LK

TI Signal peptide peptidase dependent cleavage of type II transmembrane substrates releases intracellular and extracellular signals

AU Dev K K (Reprint)

CS Novartis Pharma AG, Novartis Inst BioMed Res, CH-4002 Basel, Switzerland (Reprint)

AU Chatterjee S; Osinde M; Stauffer D; Morgan H; Kobialko M; Dengler U; Rueeger H; Martoglio B; Rovelli G

CS E-mail: kumlesh_k.dev@novartis.com

CYA Switzerland

SO EUROPEAN JOURNAL OF PHARMACOLOGY, (1 JUL 2006) Vol. 540, No. 1-3, pp. 10-17.
ISSN: 0014-2999.

PB ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.

DT Article; Journal

LA English

REC Reference Count: 28

ED Entered STN: 27 Jul 2006
Last Updated on STN: 19 Aug 2006
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The intramembrane-cleaving proteases (I-CLiPs) presenilin-1 and -2 (PS1 and PS2), signal peptide peptidase (SPP) and the Site-2 protease (S2P) catalyze critical steps in cell signaling and are implicated in diseases such as Alzheimer's disease, hepatitis C virus (HCV) infection and cholesterol homeostasis. Here we describe the development of a cellular assay based on cleavage of the transmembrane sequence of the HCV core protein precursor, releasing intra- and extra-cellular signals that represent sequential signal peptidase and SPP cleavage, respectively. We find that the SPP inhibitor (Z-LL)2-ketone (IC50=1.33 mu M) and the gamma-secretase potent inhibitors NVP-AHW700-NX (IC50=51 nM) and LY411575

(IC50=61 nM) but not DAPT dose dependently inhibited SPP but not signal peptidase cleavage. Our data confirm that type II orientated substrates, like the HCV transmembrane sequence, are sequentially cleaved by signal peptidase then SPP. This dual assay provides a powerful tool to pharmacologically analyze sequential cleavage events of signal peptidase and SPP and their regulation. (c) 2006 Elsevier B.V All rights reserved.

L11 ANSWER 12 OF 51 MEDLINE on STN DUPLICATE 10
AN 2004621543 MEDLINE
DN PubMed ID: 15596836
TI Importance of arginine 20 of the swine vesicular disease virus 2A protease for activity and virulence.
AU Inoue Toru; Alexandersen Soren; Clark Angela T; Murphy Ciara; Quan Melvyn; Reid Scott M; Sakoda Yoshihiro; Johns Helen L; Belsham Graham J
CS Department of Exotic Disease, National Institute of Animal Health, Kodaira, Tokyo, Japan.
SO Journal of virology, (2005 Jan) Vol. 79, No. 1, pp. 428-40.
Journal code: 0113724. ISSN: 0022-538X.
Report No.: NLM-PMC538687.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LA English
FS Priority Journals
EM 200501
ED Entered STN: 20 Dec 2004
Last Updated on STN: 15 Jan 2005
Entered Medline: 14 Jan 2005
AB A major virulence determinant of swine vesicular disease virus (SVDV), an Enterovirus that causes an acute vesicular disease, has been mapped to residue 20 of the 2A protease. The SVDV 2A protease cleaves the 1D-2A junction in the viral polyprotein, induces cleavage of translation initiation factor eIF4GI, and stimulates the activity of enterovirus internal ribosome entry sites (IRESs). The 2A protease from an attenuated strain of SVDV (Ile at residue 20) is significantly defective at inducing cleavage of eIF4GI and the activation of IRES-dependent translation compared to the 2A protease from a pathogenic strain (J1/73, Arg at residue 20), but the two proteases have similar 1D-2A cleavage activities (Y. Sakoda, N. Ross-Smith, T. Inoue, and G. J. Belsham, J. Virol. 75:10643-10650, 2001). Residue 20 has now been modified to every possible amino acid, and the activities of each mutant 2A protease has been analyzed. Selected mutants were reconstructed into full-length SVDV cDNA, and viruses were rescued. The rate of virus growth in cultured swine kidney cells reflected the efficiency of 2A protease activity. In experimentally infected pigs, all four of the mutant viruses tested displayed much-reduced virulence compared to the J1/73 virus but a significant, albeit reduced, level of viral replication and excretion was detected. Direct sequencing of cDNA derived from samples taken early and late in infection indicated that a gradual selection-reversion to a more efficient protease occurred. The data indicated that extensive sequence change and selection may introduce a severe bottleneck in virus replication, leading to a decreased viral load and reduced or no clinical disease.

L11 ANSWER 13 OF 51 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN
AN 2006:136357 BIOSIS
DN PREV200600132370
TI The first deletion mutation in the TSP1-6 repeat domain of ADAMTS13 leads to a secretion defect.
AU Peyvandi, Flora [Reprint Author]; Lavoretano, Silvia; Palla, Roberta;

Garagiola, Isabella; Valsecchi, Carla; Lombardi, Rossana; Canciani, Maria T.; Mannucci, Pier M.

CS Univ Milan, Maggiore Hosp, IRCCS Fdn, Fdn L Villa,A Bianchi Bonomi Hemophilia and Thrombo, Milan, Italy

SO Blood, (NOV 16 2005) Vol. 106, No. 11, Part 2, pp. 69B-70B.
Meeting Info.: 47th Annual Meeting of the American-Society-of-Hematology. Atlanta, GA, USA. December 10 -13, 2005. Amer Soc Hematol.
CODEN: BLOOAW. ISSN: 0006-4971.

DT Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)

LA English

ED Entered STN: 22 Feb 2006
Last Updated on STN: 22 Feb 2006

AB The inherited deficiency of the von Willebrand factor-cleaving protease, ADAMTS 13, is usually associated with severe forms of thrombotic thrombocytopenic purpura (TTP). Among the reported 53 mutations on ADAMTS13 gene, none has been described on TSP1-6 repeat domain. We investigated an Iranian man with history of chronic recurrent TTP and very low plasma levels of ADAMTS 13 activity (2.3%). He had his first TTP episode at the age of 21 years and 6 subsequent episodes occurred without precipitating events. Genetic analysis revealed a homozygous deletion of nucleotides 2930-2935 (GTGCCC), in exon 23 of ADAMTS13 gene, leading to the replacement of Cys977 residue by a Trp and the deletion of Ala978 and Arg979, in the TSP1-6 repeat domain. The patient's brother, homozygous for the same mutation, has no clinical manifestation of TTP so far, despite the same degree of ADAMTS 13 deficiency. The patient's parents resulted to be heterozygous. To explore the mechanism of ADAMTS13 deficiency, wild type (ADAMT13(WT)) and mutant (ADAMTS 13(del6bp)) expression vectors were transiently transfected in HEK293 and COS-7 cells. The enzymatic activity of the expressed rADAMTS13 proteins was evaluated by measuring the extent of VWF multimer degradation, using a quantitative immunoblotting assay. rADAMT13(WT) was able to degrade multimers completely (100% of activity), whereas rADAMTS13(del6bp) bad enzymatic activity reduced to similar to 10% of WT. Both WT and mutant proteases present in conditioned media and cell lysates, were analyzed by Western blot analysis, using anti-V5 monoclonal antibody against the C-terminal tag of rADAMTS13. Blots showed a dense band of similar to 190 KDa in the medium,corresponding to the secreted rADAMT13(WT) protein. Cells transfected with ADAMTS(13del6bp) showed a fainter band roughly estimated to be 5% of the WT obtained by densiometric analysis. Therefore, pulse-chase labelling experiments were performed to evaluate the presence of a secretion pathway alteration. After 60 min pulse with [S-35] methionine the maximum level of rADAMT13(WT) in conditioned media was found at 24 hours. rADAMTS13(del6bb), was minimally present after 3 hours only, no band being detected after 7 hours of chase. Differential immunofluorescence studies were per-formed using an anti-V5 monoclonal antibody against ADAMTS13 proteins and monoclonal antibodies recognizing markers of Cis-Golgi and ER. The merging studies in WT and mutant transfected cells showed that rADAMTS 13, was mostly localized in the perinuclear area, suggesting a primary localization in the Golgi apparatus, whereas rADAMTS13(del6bp), showed less intense staining diffusely throughout the cytoplasm, only a minimal amount of the mutant protein being localized in the Cis-Golgi and ER. The structural consequences of the deletion mutation in the TSP1-6 domain may cause a loss of the known antiparallel, three-stranded fold characterizing the architecture of the TSP-like domains. In these domains, each strand is capped by disulfide bonds on each end. This study suggests that the residue Cys977 could be involved in the formation of disulphide bonds responsible for a correct folding of one of the TSP1-like domains of ADAMTS13. Therefore the deletion could lead to uncorrected folding which reflects an intracellular degradation and secretion defect of the

mutant protease without any intracellular accumulation.
These results reflect the severe deficiency of ADAMTS 13 in the patient's plasma.

L11 ANSWER 14 OF 51 MEDLINE on STN DUPLICATE 11
AN 2004044470 MEDLINE
DN PubMed ID: 14744894
TI Pseudomonas keratitis: protease IV gene conservation, distribution, and production relative to virulence and other Pseudomonas proteases.
AU Caballero Armando; Thibodeaux Brett; Marquart Mary; Traidej Mullika; O'Callaghan Richard
CS Department of Microbiology, Immunology and Parasitology, Louisiana State University Health Sciences Center, New Orleans, Louisiana 70112, USA.
NC EY12961 (United States NEI NIH HHS)
SO Investigative ophthalmology & visual science, (2004 Feb) Vol. 45, No. 2, pp. 522-30.
Journal code: 7703701. ISSN: 0146-0404.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, U.S. GOV'T, P.H.S.)
LA English
FS Priority Journals
EM 200402
ED Entered STN: 28 Jan 2004
Last Updated on STN: 13 Feb 2004
Entered Medline: 12 Feb 2004
AB PURPOSE: To determine the distribution of the protease IV gene, the production of this and other proteases by multiple strains of Pseudomonas, and the virulence of a mutant specifically deficient in protease IV. METHODS: The protease IV gene was cloned, its sequence analyzed, and its chromosomal location determined by pulse-field gel electrophoresis. Three PCR reactions were used to detect the protease IV gene in 30 Pseudomonas isolates and protease production was determined by Western blot analysis, colorimetric assay, and zymography. An allelic replacement mutant deficient in the protease IV gene was analyzed for enzyme production, corneal growth, and corneal virulence. RESULTS: The protease IV gene was identified in all P. aeruginosa, but none of the non-aeruginosa strains tested. The protease IV genes of strains PA103-29 and PAO1 were in a common chromosomal site and had 98.5% sequence identity with variations occurring mainly in the promoter region. The protease IV activity of the 23 wild-type P. aeruginosa strains tested varied from 2.3 to 221.5 x 10(-3) U/mg protein in the culture supernatant. Protease IV was produced by all P. aeruginosa wild-type strains. A protease IV-deficient mutant derived from strain PA103-29 had reduced virulence compared with its parent strain and unexpectedly produced alkaline protease. CONCLUSIONS: The protease IV gene and its product are common to P. aeruginosa, but not to other Pseudomonas species. Protease IV activity varies among P. aeruginosa strains, and a mutant specifically deficient in this activity produced alkaline protease and had reduced corneal virulence.

L11 ANSWER 15 OF 51 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN
AN 2005:476582 BIOSIS
DN PREV200510268486
TI Diverse functional implications of ADAMTS13 gene mutations in patients with TTP and congenital deficiency.
AU Donadelli, Roberta [Reprint Author]; Banterla, Federica; Capoferri, Cristina; Galbusera, Miriam; Ruggeri, Zaverici M.; Bucchioni, Sara; Noris, Marina; Remuzzi, Giuseppe
CS Mario Negri Inst Pharmacol Res, I-24100 Bergamo, Italy

SO Blood, (NOV 16 2004) Vol. 104, No. 11, Part 1, pp. 149A.
Meeting Info.: 46th Annual Meeting of the American-Society-of-Hematology. San Diego, CA, USA. December 04 -07, 2004. Amer Soc Hematol.
CODEN: BLOOAW. ISSN: 0006-4971.
DT Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LA English
ED Entered STN: 16 Nov 2005
Last Updated on STN: 16 Nov 2005
AB Thrombotic thrombocytopenic purpura (TTP) is a rare disorder of small vessels that is associated with deficiency of the VWF cleaving protease ADAMTS 13, causing life-threatening disseminated microvascular thrombosis. Here we report four missense mutations (V88M, G1239V, R1219W, R1123C) in three patients with congenital ADAMTS 13 deficiency. The three subjects carrying the mutations had less than 10% normal ADAMTS 13 plasma antigen levels (measured by ELISA), whichis consistent with previously reported data showing that most ADAMTS 13 mutations in TTP patients result in impaired secretion of the protein. To evaluate whether the small amount of the mutant protease present in patients' plasma had proteolytic activity, we cloned the ADAMTS 13 cDNA in the pMT/ Bip/His Drosophila expression vector and introduced the respective mutations by directed mutagenesis technique using wild-type cDNA as a template. These wild-type and mutant constructs were stably transfected into S2 Drosophila cells under the influence of the Drosophila BiP protein signal sequence, which allows the proteinto be secreted into the medium and overcomes impaired secretion caused by themutations. The induction of the histidine-tagged ADAMTS 13 recombinants following the addition of copper was analyzed by Western blotting using an anti-hexahistidine monoclonal antibody. Equal amounts of recombinant ADAMTS 13 proteins were used to evaluate proteolytic activity of recombinant proteins by cleavage of the rVWF A1-A2-A3 substrate. The proteolytic carboxyl terminal product of about 30 kDa was visualized by Western blot with a mouse monoclonal antibodydirected against an epitope contained within the A3 domain of VWF. All the four missense mutations exhibited reduced activity: V88M:40%, G1239V: 66%, R1219W: 62% and R1123C: 64% of wild type activity. The results were also confirmed by collagen binding assay. For the the V88M mutation (located in the metalloprotease domain), decreased activity was confirmed by kinetic studies. In conclusion, mutant ADAMTS13 proteins found in patients with TTP besides defective secretion, also have reduced protease activity. The mechanisms of the deficiencywill be the matter of further studies.

L11 ANSWER 16 OF 51 MEDLINE on STN DUPLICATE 12
AN 20034777593 MEDLINE
DN PubMed ID: 12874290
TI The Staphostatin-staphopain complex: a forward binding inhibitor in complex with its target cysteine protease.
AU Filipek Renata; Rzychon Malgorzata; Oleksy Aneta; Gruca Milosz; Dubin Adam; Potempa Jan; Bochtler Matthias
CS International Institute of Molecular and Cell Biology, ul Trojdena 4, 02-109 Warsaw, Poland.
SO The Journal of biological chemistry, (2003 Oct 17) Vol. 278, No. 42, pp. 40959-66. Electronic Publication: 2003-07-21.
Journal code: 2985121R. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LA English
FS Priority Journals
OS PDB-1PXV
EM 200312

ED Entered STN: 15 Oct 2003
Last Updated on STN: 19 Dec 2003
Entered Medline: 3 Dec 2003
AB Staphostatins are the endogenous inhibitors of the major secreted cysteine proteases of Staphylococcus aureus, the staphopains. Our recent crystal structure of staphostatin B has shown that this inhibitor forms a mixed, eight-stranded beta-barrel with statistically significant similarity to lipocalins, but not to cystatins. We now present the 1.8-A crystal structure of staphostatin B in complex with an inactive mutant of its target protease. The complex is held together through extensive interactions and buries a total surface area of 2300 A2. Unexpectedly for a cysteine protease inhibitor, staphostatin B binds to staphopain B in an almost substrate-like manner. The inhibitor polypeptide chain runs through the protease active site cleft in the forward direction, with residues IG-TS in P2 to P2' positions. Both in the free and complexed forms, the P1 glycine residue of the inhibitor is in a main chain conformation only accessible to glycines. Mutations in this residue lead to a loss of affinity of the inhibitor for protease and convert the inhibitor into a substrate.

L11 ANSWER 17 OF 51 CAPLUS COPYRIGHT 2009 ACS on STN
AN 2003:416281 CAPLUS
DN 139:115648
TI ADAMTS13 gene mutation in congenital thrombotic thrombocytopenic purpura with previously reported normal VWF cleaving protease activity
AU Savasan, Sureyya; Lee, Soon-Ki; Ginsburg, David; Tsai, Han-Mou
CS Children's Hospital of Michigan, Division of Hematology/Oncology, Wayne State University, Detroit, MI, USA
SO Blood (2003), 101(11), 4449-4451
CODEN: BLOOAW; ISSN: 0006-4971
PB American Society of Hematology
DT Journal
LA English
AB Deficiency of von Willebrand factor (VWF) cleaving protease ADAMTS13 is associated with the development of thrombotic thrombocytopenic purpura (TTP). A case of congenital TTP that was previously reported to have normal ADAMTS13 activity was analyzed at the mol. level. Reanal. of plasma VWF cleaving protease activity using a different assay revealed that the patient had less than 0.1 U/L ADAMTS13 protease activity, while the parents were both partially deficient. Sequence anal. of DNA amplified by polymerase chain reaction showed that the patient was homozygous for a novel TT deletion in exon 15 of the ADAMTS13 gene resulting in a frameshift, while both parents were heterozygous for the same mutation. Taken together with other recent reports, all the cases of hereditary TTP studied by DNA sequence anal. to date appear to be due to mutations within the ADAMTS13 gene.
OSC.G 44 THERE ARE 44 CAPLUS RECORDS THAT CITE THIS RECORD (44 CITINGS)
RE.CNT 18 THERE ARE 18 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 18 OF 51 CAPLUS COPYRIGHT 2009 ACS on STN
AN 2003:174850 CAPLUS
DN 138:335598
TI Von Willebrand factor cleaving protease and ADAMTS13 mutations in childhood TTP
AU Schneppenheim, Reinhard; Budde, Ulrich; Oyen, Florian; Angerhaus, Dorothea; Aumann, Volker; Drewke, Elke; Hassenpflug, Wolf; Haberle, Johannes; Kentouche, Karim; Kohne, Elisabeth; Kurnik, Karin; Mueller-Wiefel, Dirk; Obser, Tobias; Santer, Rene; Sykora, Karl-Walter
CS Children's University Hospital, Hamburg-Eppendorf, Germany
SO Blood (2003), 101(5), 1845-1850

CODEN: BLOOAW; ISSN: 0006-4971
PB American Society of Hematology
DT Journal
LA English
AB Thrombotic thrombocytopenic purpura (TTP) is caused by the persistence of the highly reactive high-mol.-weight multimers of von Willebrand factor (VWF) due to deficiency of the specific VWF-cleaving protease (VWF-CP) ADAMTS13, resulting in microangiopathic disease. The acquired form is caused by autoantibodies against VWF-CP, whereas homozygous or compound heterozygous mutations of ADAMTS13 are responsible for recessively inherited TTP. We investigated 83 children with hemolytic or thrombocytopenic episodes with or without addnl. neurol. symptoms or renal failure. The presumed diagnosis was chronic idiopathic thrombocytopenic purpura (ITP; n = 50), TTP (n = 8), hemolytic uremic syndrome (HUS; n = 24), and Evans syndrome (n = 1). A severe deficiency of VWF-CP (≤ 5%) was found in all investigated patients with TTP and in none of those with HUS. Addnl., 2 of 50 patients with a prior diagnosis of ITP were deficient for VWF-CP. Antibodies against VWF-CP were found in 4 children. Mutation anal. of the ADAMTS13 gene in the patients deficient in VWF-CP by direct sequencing of all 29 exons identified 8 different mutations, suggesting the hereditary form of TTP in 1 patient with ITP, in the patient with Evans syndrome, and in 5 of the 8 patients with TTP. The phenotype of TTP in childhood can be rather variable. Besides the classical clin. picture, oligosymptomatic forms may occur that can delay the identification of patients at risk.
OSC.G 73 THERE ARE 73 CAPLUS RECORDS THAT CITE THIS RECORD (73 CITINGS)
RE.CNT 29 THERE ARE 29 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 19 OF 51 MEDLINE on STN DUPLICATE 13
AN 2003327749 MEDLINE
DN PubMed ID: 12858075
TI An update on the pathogenesis and management of acquired thrombotic thrombocytopenic purpura.
AU Yarranton Helen; Machin Samuel J
CS Haemostasis Research Unit, Department of Haematology, University College London, London, UK.
SO Current opinion in neurology, (2003 Jun) Vol. 16, No. 3, pp. 367-73. Ref: 48
Journal code: 9319162. ISSN: 1350-7540.
CY England: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
LA English
FS Priority Journals
EM 200308
ED Entered STN: 15 Jul 2003
Last Updated on STN: 16 Aug 2003
Entered Medline: 15 Aug 2003
AB PURPOSE OF REVIEW: Thrombotic thrombocytopenic purpura, a clinical syndrome characterized by thrombocytopenia and microangiopathic haemolytic anaemia, was almost universally fatal until the introduction of plasma exchange therapy in the 1970s. Current outcomes have improved dramatically with the initiation of prompt plasma exchange, a treatment routinely used without any real understanding of why it is effective. RECENT FINDINGS: Recent advances suggest that a deficiency of a specific plasma metalloprotease, responsible for the physiological processing of von Willebrand factor multimers, plays a substantial role in the pathogenesis of congenital and acquired idiopathic thrombotic thrombocytopenic purpura. The von Willebrand factor-cleaving protease has now been identified as a new member of the ADAMTS family of metalloproteases, designated ADAMTS13. The acquired form of

thrombotic thrombocytopenic purpura is associated with inhibitory autoantibodies against ADAMTS13, and the congenital chronic relapsing form is caused by mutations in the ADAMTS13 gene, resulting in a constitutional deficiency. Plasma exchange has been proved to be the most important therapy in thrombotic thrombocytopenic purpura, but clinical data for adjunctive therapies, such as corticosteroids, antiplatelet drugs and other immunosuppressive agents often used in combination with plasma exchange, are less well defined. SUMMARY: Recent advances in our understanding of the pathological mechanisms of thrombotic thrombocytopenic purpura not only provide a rationale for the previously empirical plasma exchange therapy (removal of the inhibitory antibodies and replacement of the deficient protease from the plasma infused), but may also help in developing more rational and targeted treatment strategies. This review discusses the clinical presentation, pathophysiology and current management of thrombotic thrombocytopenic purpura.

L11 ANSWER 20 OF 51 CAPLUS COPYRIGHT 2009 ACS on STN
AN 2003:294313 CAPLUS
DN 139:50682
TI TTP and ADAMTS13 mutation
AU Fujimura, Yoshihiro
CS Affiliated Hospital, Nara Prefectural Medical University, Japan
SO Annual Review Ketsueki (2003) 153-162
CODEN: ARKNB7
PB Chugai Igakusha
DT Journal; General Review
LA Japanese
AB A review on von Willebrand factor (vWF) cleaving protease ADAMTS13 mutation in thrombotic thrombocytopenic purpura (TTP). The topics discussed are (1) unusually large vWF multimers in TTP; (2) vWF cleaving protease activity and its IgG type inhibitor; (3) TTP vs. Upshaw-Schulman syndrome; and (4) von Willebrand factor cleaving protease ADAMTS13 and its mutation in TTP.

L11 ANSWER 21 OF 51 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN
AN 2002:558135 BIOSIS
DN PREV200200558135
TI Mutations and common polymorphisms in ADAMTS13 gene responsible for von Willebrand factor-cleaving protease activity.
AU Kokame, Koichi; Matsumoto, Masanori; Soejima, Kenji; Yagi, Hideo; Ishizashi, Hiromichi; Funato, Masahisa; Tamai, Hiroshi; Konno, Mutsuko; Kamide, Kei; Kawano, Yuhei; Miyata, Toshiyuki [Reprint author]; Fujimura, Yoshihiro
CS Research Institute, National Cardiovascular Center, Suita, Osaka, 565-8565, Japan
miyata@ri.ncvc.go.jp
SO Proceedings of the National Academy of Sciences of the United States of America, (September 3, 2002) Vol. 99, No. 18, pp. 11902-11907. print.
CODEN: PNASA6. ISSN: 0027-8424.
DT Article
LA English
ED Entered STN: 30 Oct 2002
Last Updated on STN: 30 Oct 2002
AB von Willebrand factor (VWF) is synthesized primarily in vascular endothelial cells and secreted into the plasma as unusually large VWF multimers. Normally, these multimers are quickly degraded into smaller forms by a plasma metalloproteinase, VWF-cleaving protease (VWF-CP). Decreases in the activity of this enzyme result in congenital and acquired

thrombotic thrombocytopenic purpura (TTP). The human VWF-CP has recently been purified. Cloning of the corresponding cDNA revealed that the 1,427-aa polypeptide is a member of the ADAMTS gene family, termed ADAMTS13. Twelve rare mutations in this gene have been identified in patients with congenital TTP. Here, we report missense and nonsense mutations in two Japanese families with Upshaw-Schulman syndrome, congenital TTP with neonatal onset and frequent relapses. The comparison of individual ADAMTS13 genotypes and plasma VWF-CP activities indicated that the R268P, Q449stop, and C508Y mutations abrogated activity of the enzyme, whereas the P475S mutant retained low but significant activity. The effects of these mutations were further confirmed by expression analysis in HeLa cells. Recombinant VWF-CP containing either the R268P or C508Y mutations was not secreted from cells. In contrast, Q449stop and P475S mutants were normally secreted but demonstrated minimal activity. Genotype analysis of 364 Japanese subjects revealed that P475S is heterozygous in 9.6% of individuals, suggesting that approximately 10% of the Japanese population possesses reduced VWF-CP activity. We report on a single-nucleotide polymorphism associated with alterations in VWF-CP activity; it will be important to assess this single-nucleotide polymorphism as a risk factor for thrombotic disorders.

L11 ANSWER 22 OF 51 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN
AN 2002:369748 BIOSIS
DN PREV200200369748
TI Novel mechanism of activity control of potyvirus NIa protease.
AU Han, Jisun [Reprint author]; Kang, Hyun-Jin [Reprint author]; Song, Byeong-Doo; Choi, Kwan-Yong [Reprint author]
CS Life Science, Pohang University of Science and Technology, Hyo-ja Dong San 31, Pohang, Kyungbuk, 790-784, South Korea
SO FASEB Journal, (March 22, 2002) Vol. 16, No. 5, pp. A905. print.
Meeting Info.: Annual Meeting of Professional Research Scientists on Experimental Biology. New Orleans, Louisiana, USA. April 20-24, 2002.
CODEN: FAJOEC. ISSN: 0892-6638.
DT Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LA English
ED Entered STN: 3 Jul 2002
Last Updated on STN: 3 Jul 2002
AB Potato virus a (PVA) is a member of the potyvirus that belongs to the picornavirus superfamily of positive-stranded RNA viruses. The viral genome has one open reading flame that is translated into a polyprotein. The nuclear inclusion protein a (NIa) protease plays a key role in the potyviral life cycle by processing the viral polyprotein into functional proteins in cis or in trans. Among seven cleavage sites of NIa protease, the peptides representing VPg/NIaPro junction (V peptide) and NIa/NIb junction (E peptide) were processed only in cis. Investigation of the kinetics of NIa protease-peptide interactions revealed that the V peptide and E peptide acted like a competitive inhibitor for the reaction in which the peptide representing NIb/CP junction was used as a substrate. These results indicate that the peptides containing the cis-cleavage site sequences can strongly bind to the active site of the protease but are not cleaved by the enzyme. Kinetic analyses of mutant NIa protease combined with in vitro translation experiments suggest that the active site of the NIa protease is no longer occupied with the C-terminal four amino acids (P1-P4) after the cis-cleavage reaction between the NIa and NIb.

L11 ANSWER 23 OF 51 CAPLUS COPYRIGHT 2009 ACS on STN
AN 2002:526312 CAPLUS
DN 138:219418

TI Predicting response to plasma exchange in patients with thrombotic thrombocytopenic purpura with measurement of vWF-cleaving protease activity

AU Mori, Yoshitaka; Wada, Hideo; Gabazza, Esteban C.; Minami, Nobuyuki; Nobori, Tsutomu; Shiku, Hiroshi; Yagi, Hideo; Ishizashi, Hiromichi; Matsumoto, Masanori; Fujimura, Yoshihiro

CS Mie Red Cross Blood Center, Department of Clinical Laboratory and Second Department of Internal Medicine, Mie University School of Medicine, Tsu City, 514-8507, Japan

SO Transfusion (Malden, MA, United States) (2002), 42(5), 572-580
CODEN: TRANAT; ISSN: 0041-1132

PB Blackwell Publishing, Inc.

DT Journal

LA English

AB Severe deficiency of vWF-cleaving protease (vWF-CPase) activity was recently found in patients with thrombotic thrombocytopenic purpura (TTP). Although the survival of patients with TTP has been dramatically improved with plasma exchange (PE), there are still many patients who are refractory to PE and immunosuppressive therapy. The activities of vWF-CPase and its inhibitor were measured in 27 patients with nonfamilial TTP and hemolytic-uremic syndrome (HUS) to examine the relationship between the clin. variables and vWF-CPase activity. Eight of nine patients with HUS had more than 40 % of vWF-CPase activity, whereas one had 28 % of the normal level at the acute phase. Ten of 12 TTP patients with a good outcome had a severe deficiency of vWF-CPase activity and its inhibitor, whereas four of six patients with a poor outcome had a moderate deficiency of vWF-CPase activity along with a lack of the inhibitor. PE produced normalization of the vWF-CPase activity and neutralization of the inhibitor in TTP patients with a good outcome; however, some TTP patients with vWF-CPase inhibitor had relapsed and required an immunosuppressive therapy. The response to the combination therapy with PE and immunosuppressive treatment was poor in TTP patients without a severe deficiency of vWF-CPase activity. Assays of vWF-CPase activity and its inhibitor may be useful for predicting the response to therapy and the outcome of patients with TTP. In some patients, nonfamilial TTP with a poor prognosis may not be caused by a constitutional or acquired deficiency of vWF-CPase with its inhibitor. Although PE and immunosuppressive therapy are effective in patients with nonfamilial TTP and a vWF-CPase inhibitor, other therapeutic modalities may be needed for nonfamilial TTP with unknown etiol.

OSC.G 37 THERE ARE 37 CAPLUS RECORDS THAT CITE THIS RECORD (37 CITINGS)

RE.CNT 39 THERE ARE 39 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 24 OF 51 MEDLINE on STN DUPLICATE 14

AN 2003023698 MEDLINE

DN PubMed ID: 12530635

TI Identification of two alternate splice variants of a novel serine protease expressed in steroidogenic tissues.

AU Omer Selma; Lomthaisong Khomsorn; Bicknell Andrew B

CS School of Animal and Microbial Sciences, The University of Reading, Whiteknights, P.O. Box 228, Reading RG6 6AJ, UK.

SO Endocrine research, (2002 Nov) Vol. 28, No. 4, pp. 339-48.
Journal code: 8408548. ISSN: 0743-5800.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 200307

ED Entered STN: 18 Jan 2003
Last Updated on STN: 17 Jul 2003

Entered Medline: 16 Jul 2003
AB During the search for the serine protease that cleaves pro-gamma-melatropin to stimulate adrenal growth, we identified another novel protease, which we called Adrenal mitochondrial protease (AmP). In situ hybridisation detected AmP transcripts in steroidogenic tissues such as the brain, testis, in ovarian follicles as well as in the adrenal cortex. Full length cloning identified two splice variants differing by a 222 nucleotide insertion in the 5' end of the short variant. The shorter variant codes for a 371 amino acid protein of 40.7 kDa and computer analysis predicts it to be targeted to the cytosol while the longer 445 amino acid protein of 48.4 kDa is mitochondrial. Cellular targeting was confirmed by tagging with GFP. The short variant was clearly cytosolic however, the cells expressing AmP-Long had large vacuoles, possibly as a result of distended (apoptotic?) mitochondria. Due to the mitochondrial localisation of the long variant of the protease and its expression in steroidogenic tissues, it may be expected to be involved in the steroidogenic pathway, possibly by cleaving steroidogenic acute regulatory protein (StAR). We investigated this by co-transfecting AmP-Long with StAR and F2 plasmid into COS-1 cells and measuring the effect on pregnenolone production. It was found that AmP-Long has no effect on steroidogenesis nor cleaves StAR as was shown by western blot analysis using StAR antibody.

L11 ANSWER 25 OF 51 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN
AN 2002:545058 BIOSIS
DN PREV200200545058
TI Proteases acting on mutant huntingtin generate cleaved products that differentially build up cytoplasmic and nuclear inclusions.
AU Lunkes, Astrid [Reprint author]; Lindenberg, Katrin S.; Ben-Haiem, Lea; Weber, Chantal; Devys, Didier; Landwehrmeyer, G. Bernhard; Mandel, Jean-Louis; Trottier, Yvon
CS European Molecular Biology Organization, Meyerhofstrasse 1, 69117, Heidelberg, Germany
astrid.lunkes@embo.org; yvon@igbmc.u-strasbg.fr
SO Molecular Cell, (August, 2002) Vol. 10, No. 2, pp. 259-269. print.
ISSN: 1097-2765.
DT Article
LA English
ED Entered STN: 23 Oct 2002
Last Updated on STN: 23 Oct 2002
AB Proteolytic processing of mutant huntingtin (mhtt) is regarded as a key event in the pathogenesis of Huntington's disease (HD). Mhtt fragments containing a polyglutamine expansion form intracellular inclusions and are more cytotoxic than full-length mhtt. Here, we report that two distinct mhtt fragments, termed cp-A and cp-B, differentially build up nuclear and cytoplasmic inclusions in HD and in a cellular model for HD. Cp-A is released by cleavage of htt in a 10 amino acid domain and is the major fragment that aggregates in the nucleus. Furthermore, we provide evidence that cp-A and cp-B are most likely generated by aspartic endopeptidases acting in concert with the proteasome to ensure the normal turnover of htt. These proteolytic processes are thus potential targets for therapeutic intervention in HD.

L11 ANSWER 26 OF 51 MEDLINE on STN DUPLICATE 15
AN 2002334152 MEDLINE
DN PubMed ID: 12077234
TI Exogenous peptides delivered by ricin require processing by signal peptidase for transporter associated with antigen processing-independent MHC class I-restricted presentation.
AU Smith Daniel C; Gallimore Awen; Jones Emma; Roberts Brenda; Lord J

Michael; Deeks Emma; Cerundolo Vincenzo; Roberts Lynne M
CS Department of Biological Sciences, University of Warwick, Coventry, United Kingdom.
SO Journal of immunology (Baltimore, Md. : 1950), (2002 Jul 1) Vol. 169, No. 1, pp. 99-107.
Journal code: 2985117R. ISSN: 0022-1767.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 200208
ED Entered STN: 23 Jun 2002
Last Updated on STN: 14 Aug 2002
Entered Medline: 13 Aug 2002
AB In this study we demonstrate that a disarmed version of the cytotoxin ricin can deliver exogenous CD8(+) T cell epitopes into the MHC class I-restricted pathway by a TAP-independent, signal peptidase-dependent pathway. Defined viral peptide epitopes genetically fused to the N terminus of an attenuated ricin A subunit (RTA) that was reassociated with its partner B subunit were able to reach the early secretory pathway of sensitive cells, including TAP-deficient cells. Successful processing and presentation by MHC class I proteins was not dependent on proteasome activity or on recycling of MHC class I proteins, but rather on a functional secretory pathway. Our results demonstrated a role for signal peptidase in the generation of peptide epitopes associated at the amino terminus of RTA. We showed, first, that potential signal peptide cleavage sites located toward the N terminus of RTA can be posttranslationally cleaved by signal peptidase and, second, that
mutation of one of these sites led to a loss of peptide
presentation. These results identify a novel MHC class I presentation pathway that exploits the ability of toxins to reach the lumen of the endoplasmic reticulum by retrograde transport, and suggest a role for endoplasmic reticulum signal peptidase in the processing and presentation of MHC class I peptides. Because TAP-negative cells can be sensitized for CTL killing following retrograde transport of toxin-linked peptides, application of these results has direct implications for the development of novel vaccination strategies.

L11 ANSWER 27 OF 51 SCISEARCH COPYRIGHT (c) 2009 The Thomson Corporation on STN
AN 2003:572784 SCISEARCH
GA The Genuine Article (R) Number: 614JK
TI Determination of von Willebrand factor cleaving protease
and ADAMTS13 mutation screening in the differential diagnosis of hemolytic-thrombocytopenic disorders in childhood.
AU Schneppenheim R (Reprint); Budde U; Oyen F; Angerhaus D; Aumann V; Drewke E; Hassenpflug W A; Haberle J; Kentouche K; Kohne E; Kurnik K; Mueller-Wiefel D E; Obser T; Santer R; Sykora K W
CS Childrens Univ Hosp, Hamburg, Germany; Lab Assoc Prof Arndt & Partners, Coagulat Lab, Hamburg, Germany; Childrens Univ Hosp, Magdeburg, Germany; Childrens Univ Hosp, Munster, Germany; Childrens Univ Hosp, Jena, Germany; Childrens Univ Hosp, Ulm, Germany; Childrens Univ Hosp, Munich, Germany; Childrens Univ Hosp, Kiel, Germany; Hannover Med Sch, D-3000 Hannover, Germany
CYA Germany
SO BLOOD, (16 NOV 2002) Vol. 100, No. 11, Part 1, pp. 56A-56A. MA 201. ISSN: 0006-4971.
PB AMER SOC HEMATOLOGY, 1900 M STREET. NW SUITE 200, WASHINGTON, DC 20036 USA.
DT Conference; Journal

LA English
REC Reference Count: 0
ED Entered STN: 25 Jul 2003
Last Updated on STN: 25 Jul 2003

L11 ANSWER 28 OF 51 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN
AN 2003:337362 BIOSIS
DN PREV200300337362
TI Determination of Von Willebrand Factor Cleaving Protease and ADAMTS13 Mutation Screening in the Differential Diagnosis of Hemolytic-Thrombocytopenic Disorders in Childhood.
AU Schneppenheim, Reinhard [Reprint Author]; Budde, Ulrich [Reprint Author]; Oyen, Florian [Reprint Author]; Angerhaus, Dorthe [Reprint Author]; Aumann, Volker [Reprint Author]; Drewke, Elke [Reprint Author]; Hassenpflug, Wolf A. [Reprint Author]; Haberle, Johannes [Reprint Author]; Kentouche, Karim [Reprint Author]; Kohne, Elisabeth [Reprint Author]; Kurnik, Karin [Reprint Author]; Mueller-Wiefel, Dirk E. [Reprint Author]; Obser, Tobias [Reprint Author]; Santer, Rene [Reprint Author]; Sykora, Karl-Walter [Reprint Author]
CS Pediatric Hematology and Oncology, Children's University Hospital Hamburg, Hamburg, Germany
SO Blood, (November 16 2002) Vol. 100, No. 11, pp. Abstract No. 201. print. Meeting Info.: 44th Annual Meeting of the American Society of Hematology. Philadelphia, PA, USA. December 06-10, 2002. American Society of Hematology.
CODEN: BLOOAW. ISSN: 0006-4971.
DT Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LA English
ED Entered STN: 23 Jul 2003
Last Updated on STN: 23 Jul 2003
AB Thrombotic thrombocytopenic purpura (TTP), a microangiopathic disease, is caused by the persistence of the highly reactive high molecular weight multimers of von Willebrand factor (VWF) due to deficiency of the specific VWF cleaving protease (VWF-CP), ADAMTS13. The acquired form is caused by autoantibodies against VWF-CP, whereas homozygous or compound heterozygous mutations of ADAMTS13 are responsible for recessively inherited TTP. We investigated 83 children with hemolytic and/or thrombocytopenic episodes with or without additional neurologic symptoms or renal failure. The presumed diagnoses were chronic ITP (n=50), TTP (n=8), HUS (n=24) and Evans syndrome (n=1). Eleven patients showed a severe deficiency of VWF-CP (ltoreq5 %). In addition to the 8 patients with a correct diagnosis of TTP, two were first diagnosed to have ITP and one with Evans syndrome. No patient with HUS displayed VWF-CP values <62 %. Antibodies against VWF-CP were found in 4 children. Mutation analysis of the ADAMTS13 gene in the VWF-CP deficient patients by direct sequencing of all 29 exons identified 14 mutations in 7 unrelated individuals. These were three missense mutations, L232Q (n=1), S263C (n=2), P353L (n=2), three nonsense mutations W390X (n=1), R910X (n=2), R1034X (n=1), one two base frameshift deletion 2549-2550delAT (n=1), and one single base frameshift insertion, 4143insA that was the most frequent in our cohort (n=4). Except one homozygous patient with the mutation S263C, all were compound-heterozygous. None of these mutations has been described before, neither were they found on 100 chromosomes of normal controls. These data suggest the diagnosis of hereditary VWF-CP deficiency in one of the patients previously diagnosed with ITP, in the patient with the previous diagnosis of Evans syndrome, and in 5 of the 8 patients with TTP. Molecular analysis of the specific VWF cleaving site by sequencing the VWF A2 domain coding region in the patients with HUS and TTP did not identify any mutation that could confer resistance of VWF to VWF-CP. In

conclusion, the phenotype of TTP in childhood can be rather variable. Besides the classical clinical picture, abortive forms may occur that can delay the identification of patients at risk. Determination of VWF-CP and ADAMTS13 mutation screening is helpful in the differential diagnosis of diseases characterized by recurrent hemolytic and/or thrombocytopenic episodes and to discriminate between the acquired and the hereditary form of TTP.

L11 ANSWER 29 OF 51 MEDLINE on STN DUPLICATE 16
AN 2001349845 MEDLINE
DN PubMed ID: 11413376
TI Mutagenesis of the dengue virus type 2 NS3 proteinase and the production of growth-restricted virus.
AU Matusan A E; Kelley P G; Pryor M J; Whisstock J C; Davidson A D; Wright P J
CS Department of Microbiology and Department of Biochemistry and Molecular Biology, Monash University, PO Box 53, Victoria 3800, Australia.
SO The Journal of general virology, (2001 Jul) Vol. 82, No. Pt 7, pp. 1647-56.
Journal code: 0077340. ISSN: 0022-1317.
CY England: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LA English
FS Priority Journals
EM 200108
ED Entered STN: 13 Aug 2001
Last Updated on STN: 13 Aug 2001
Entered Medline: 9 Aug 2001
AB The N-terminal one-third of the NS3 protein of Dengue virus type 2 (DEN-2) complexes with co-factor NS2B to form an active serine proteinase which cleaves the viral polyprotein. To identify sites within NS3 that may interact with NS2B, seven regions within the NS3 proteinase outside the conserved flavivirus enzyme motifs were mutated by alanine replacement. Five sites contained clusters of charged residues and were hydrophilic. Two sites were hydrophobic and highly conserved among flaviviruses. The effects of five mutations on NS2B/3 processing were examined using a COS cell expression system. Four retained significant proteinase activity. Three of these mutations and two more were introduced into genomic-length cDNA and tested for their effects on virus replication. The five mutant viruses showed reduced plaque size and two of the five showed significantly reduced titres. All seven mutations were mapped on the X-ray crystal structure of the DEN-2 NS3 proteinase: three were located at the N terminus and two at the C terminus of the NS2B-binding cleft. Two mutations were at the C terminus of the proteinase domain and one was solvent-exposed. The study demonstrated that charged-to-alanine mutagenesis in the viral proteinase can be used to produce growth-restricted flaviviruses that may be useful in the production of attenuated vaccine strains.

L11 ANSWER 30 OF 51 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN
AN 2001:317274 BIOSIS
DN PREV200100317274
TI Persistent inhibition of von Willebrand factor cleaving protease predicts a prolonged course and relapse in patients with thrombotic thrombocytopenic purpura.
AU Fijnheer, R. [Reprint author]; Hene, R. J.; Schiphorst, M. E. [Reprint author]; Sixma, J. J. [Reprint author]
CS Haematology, University Medical Center Utrecht, Utrecht, Netherlands
SO Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 631a. print.

Meeting Info.: 42nd Annual Meeting of the American Society of Hematology. San Francisco, California, USA. December 01-05, 2000. American Society of Hematology.
CODEN: BLOOAW. ISSN: 0006-4971.
DT Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
Conference; (Meeting Poster)
LA English
ED Entered STN: 4 Jul 2001
Last Updated on STN: 19 Feb 2002
AB Introduction: Thrombotic thrombocytopenic purpura (TTP) is characterized by the inhibition of a plasma cleaving protease that specifically cleaves von Willebrand factor (vWF cleaving protease), thus reducing its multimeric size. We performed a study in 9 patients with TTP and studied the course of inhibition of vWF cleaving protease activity compared to the clinical outcome. Methods: Plasma from patients with TTP was incubated with recombinant vWF R834Q, a vWF mutant with enhanced sensitivity to the protease. The proteolysis of vWF multimers was analyzed and quantified on Western blot and by collagen binding capacity. The vWF cleaving protease was measured before the start, after 1-2 cycles of plasma exchange and after 3 months. Results: vWF cleaving protease activity was inhibited, before the start of plasma exchange, in all patients (0%, range 0-4%). After initial plasma exchange, the vWF cleaving protease activity was normalized in 3 patients (102%, range 88-144%) and these patients had a quick recovery of peripheral blood counts. Persistent inhibition of vWF cleaving protease (2%, range 0-20%) was correlated with a prolonged course and multiple relapses. Four patients remained to have a complete lack of vWF cleaving protease activity even in period of normal blood counts and no need for plasma exchange. Conclusion: vWF cleaving protease activity predicts the clinical course of TTP and persistent inhibition points at patients with relapsing TTP. On the other hand patients with a totally inhibited vWF cleaving protease can have normal blood counts. We hypothesize that in the etiology of TTP, inhibition of vWF cleaving protease is not enough and a second hit is probably necessary.

L11 ANSWER 31 OF 51 SCISEARCH COPYRIGHT (c) 2009 The Thomson Corporation on STN
AN 1999:500666 SCISEARCH
GA The Genuine Article (R) Number: 212BK
TI Mechanism of endoplasmic reticulum retention of mutant vasopressin precursor caused by a signal peptide truncation associated with diabetes insipidus
AU Spiess M (Reprint)
CS Univ Basel, Bioctr, Klingelbergstr 70, CH-4056 Basel, Switzerland (Reprint)
AU Beuret N; Rutishauser J; Bider M D
CS Univ Basel, Bioctr, CH-4056 Basel, Switzerland; Kantonsspital, Dept Med, CH-6000 Luzern 16, Switzerland
CYA Switzerland
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (2 JUL 1999) Vol. 274, No. 27, pp. 18965-18972.
ISSN: 0021-9258.
PB AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814 USA.
DT Article; Journal
LA English
REC Reference Count: 42

ED Entered STN: 1999
Last Updated on STN: 1999
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Autosomal dominant neurohypophyseal diabetes insipidus is caused by mutations in the gene encoding the vasopressin precursor protein, prepro-vasopressin-neurophysin II. We analyzed the molecular consequences of a mutation (Delta G227) recently identified in a Swiss kindred that destroys the translation initiation codon, In COS-7 cells transfected with the mutant cDNA, translation was found to initiate at an alternative ATG, producing a truncated signal sequence that was functional for targeting and translocation but was not cleaved by signal peptidase, The mutant precursor was completely retained within the endoplasmic reticulum. The uncleaved signal did not affect folding of the neurophysin portion of the precursor, as determined by its protease resistance. However, formation of disulfide-linked aggregates indicated that it interfered with the formation of the disulfide bond in vasopressin, most likely by blocking its insertion into the hormone binding site of neurophysin. Preventing disulfide formation in the vasopressin nonapeptide by mutation of cysteine 6 to serine was shown to be sufficient to cause aggregation and retention. These results indicate that the Delta G227 mutation induces translation of a truncated signal sequence that cannot be cleaved but prevents correct folding and oxidation of vasopressin, thereby causing precursor aggregation and retention in the endoplasmic reticulum.

L11 ANSWER 32 OF 51 MEDLINE on STN DUPLICATE 17
AN 1999428772 MEDLINE
DN PubMed ID: 10497121
TI Second site mutations in the N-terminus of the major capsid protein (VP5) overcome a block at the maturation cleavage site of the capsid scaffold proteins of herpes simplex virus type 1.
AU Desai P; Person S
CS Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, USA.
NC AI33077 (United States NIAID NIH HHS)
SO Virology, (1999 Sep 1) Vol. 261, No. 2, pp. 357-66.
Journal code: 0110674. ISSN: 0042-6822.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, U.S. GOV'T, P.H.S.)
LA English
FS Priority Journals
EM 199910
ED Entered STN: 26 Oct 1999
Last Updated on STN: 18 Dec 2002
Entered Medline: 12 Oct 1999
AB VP5, the major capsid protein of herpes simplex virus type 1 (HSV-1), interacts with the C-terminal residues of the scaffold molecules encoded by the overlapping UL26 and UL26.5 open reading frames. Scaffold molecules are cleaved by a UL26 encoded protease (VP24) as part of the normal capsid assembly process. In this study, residues of VP5 have been identified that alter its interaction with the C-terminal residues of the scaffold proteins. A previously isolated virus (KUL26-610/611) was used that encoded a lethal mutation in the UL26 and UL26.5 open reading frames and required a transformed cell line that expresses these proteins for virus growth. The scaffold maturation cleavage site between amino acids 610 and 611 was blocked by changing Ala-Ser to Glu-Phe, which generated a new EcoRI restriction site. Revertant viruses, that formed small plaques on nontransformed cells, were detected at a frequency of 1:3800. Nine revertants were isolated, and all of them retained the EcoRI site and therefore were due to mutations at a

second site. The second site mutations were extragenic. Using marker-transfer techniques, the mutation in one of the revertants was mapped to the 5' region of the gene encoding VP5. DNA sequence analysis was performed for the N-terminal 571 codons encoding VP5 for all of the revertant viruses. Six of the nine revertants showed a single base pair change that caused an amino acid substitution between residues 30 and 78 of VP5. Three of these were identical and changed Ala to Val at residue 78. The data provide a partial map of residues of VP5 that alter its interaction with scaffold proteins blocked at their normal cleavage site. The yeast two-hybrid system was used as a measure of the interaction between mutant VP5 and scaffold molecules and varied from 11% to nearly 100%, relative to wild-type VP5. One revertant gave no detectable interaction by this assay. The amount of UL26 encoded protease (VP24) in B capsids for KUL26-610/611 and for revertants was 7% and 25%, respectively, relative to the amount in capsids for wild-type virus. The lack of retention of the viral protease in the mutant virus and a fourfold increase for the revertants suggest an additional essential function for VP24 in capsid maturation, and a role in DNA packaging is indicated.

L11 ANSWER 33 OF 51 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN
AN 1999:214234 BIOSIS
DN PREV199900214234
TI Recovery and half-life of von Willebrand factor (vWF)-cleaving protease after plasma exchange therapy in patients with thrombotic thrombocytopenic purpura (TTP).
AU Furlan, M. [Reprint author]; Robles, R. [Reprint author]; Morselli, B.; Sandoz, P.; Lammle, B. [Reprint author]
CS Central Hematology Laboratory, University Hospital, Inselspital, Bern, Switzerland
SO Annals of Hematology, (1999) Vol. 78, No. SUPPL. 1, pp. A17. print.
Meeting Info.: 43rd Annual Meeting of the Society for Thrombosis and Hemostasis. Mannheim, Germany. February 24-27, 1999. Society for Thrombosis and Hemostasis.
ISSN: 0939-5555.
DT Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LA English
ED Entered STN: 26 May 1999
Last Updated on STN: 26 May 1999

L11 ANSWER 34 OF 51 CAPLUS COPYRIGHT 2009 ACS on STN
AN 1998:485878 CAPLUS
DN 129:227431
OREF 129:46169a,46172a
TI Engineering the S1' subsite of trypsin: Design of a protease which cleaves between dibasic residues
AU Kurth, Torsten; Grahn, Sibylla; Thormann, Michael; Ullmann, Dirk; Hofmann, Hans-Joerg; Jakubke, Hans-Dieter; Hedstrom, Lizbeth
CS Department of Biochemistry, Brandeis University, Waltham, MA, 02454, USA
SO Biochemistry (1998), 37(33), 11434-11440
CODEN: BICHAW; ISSN: 0006-2960
PB American Chemical Society
DT Journal
LA English
AB Here, trypsin was redesigned by protein engineering into a site-specific protease which hydrolyzes peptides between dibasic residues. Trypsin normally exhibits high S1 specificity for Arg and Lys residues. However, the S1' specificity of trypsin is very broad, with only a slight

preference for hydrophobic residues in P1'. Here, the authors replaced Lys-60 with Glu and Asp to introduce a high specificity for basic residues into the S1' site of trypsin. Both mutations caused a dramatic increase in S1' specificity for Arg and Lys as measured by acyl transfer reactions. In mutant K60E, the preference for Arg increased 70-fold, whereas the preference for P1'-Lys increased 12-fold. In contrast, the preferences for other P1' residues either decreased slightly or remained the same. Thus, K60E prefers P1'-Arg over most other P1' residues by 2 orders of magnitude. Similar results were obtained when P1' specificity was measured in peptide cleavage assays. K60D exhibited an S1' specificity profile very similar to that of K60E, although the P1'-Arg preference was reduced by a factor of 2.5. Mol. modeling studies suggested that the high S1' specificity for Arg in K60E may be due to the formation of a salt bridge between Glu-60 and P1'-Arg of the substrate.

OSC.G 22 THERE ARE 22 CAPLUS RECORDS THAT CITE THIS RECORD (22 CITINGS)
RE.CNT 25 THERE ARE 25 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 35 OF 51 MEDLINE on STN DUPLICATE 18
AN 1998362178 MEDLINE
DN PubMed ID: 9696869
TI Molecular characterization of proteolytic processing of the Pol proteins of human foamy virus reveals novel features of the viral protease.
AU Pfrepper K I; Rackwitz H R; Schnolzer M; Heid H; Lochelt M; Flugel R M
CS Abteilungen Retroviral Gene Expression, Research Program Applied Tumorvirology, German Cancer Research Center, 69009 Heidelberg, Federal Republic of Germany.
SO Journal of virology, (1998 Sep) Vol. 72, No. 9, pp. 7648-52.
Journal code: 0113724. ISSN: 0022-538X.
Report No.: NLM-PMC110030.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199809
ED Entered STN: 25 Sep 1998
Last Updated on STN: 3 Mar 2000
Entered Medline: 16 Sep 1998
AB Spumaviruses, or foamy viruses, express a pol-specific transcript that codes for a Pol polyprotein that consists of the protease, reverse transcriptase, ribonuclease H, and the integrase domains. To delineate the proteolytic cleavage sites between the Pol subdomains, recombinant human foamy virus (HFV) Pol proteins were expressed, purified by affinity chromatography, and subjected to either HFV protease assays or autocatalytic processing. In control experiments, HFV protease -deficient mutant proteins in which the active site Asp was replaced by an Ala residue were used to rule out unspecific processing by nonviral proteases. Specific proteolytic cleavage products were isolated, and the cleavage sites were analyzed by amino acid sequencing. Peptides spanning the resulting cleavage sites were chemically synthesized and assayed with HFV protease, and the cleaved peptides were subjected to mass spectrometry. The cleavage site sequences obtained were in complete agreement with the amino-terminal sequences from amino acid sequencing of authentic cleavage products of the HFV Pol proteins. Analysis by fast-protein liquid chromatography of a short version of the active HFV protease revealed that the enzyme predominantly formed dimeric molecules.

L11 ANSWER 36 OF 51 MEDLINE on STN DUPLICATE 19
AN 1998422118 MEDLINE
DN PubMed ID: 9751498

TI A protease-resistant form of insulin-like growth factor (IGF) binding protein 4 inhibits IGF-1 actions.
AU Rees C; Clemmons D R; Horvitz G D; Clarke J B; Busby W H
CS Department of Medicine, University of North Carolina School of Medicine, Chapel Hill 27599-7170, USA.
NC HL-56850 (United States NHLBI NIH HHS)
SO Endocrinology, (1998 Oct) Vol. 139, No. 10, pp. 4182-8.
Journal code: 0375040. ISSN: 0013-7227.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, U.S. GOV'T, P.H.S.)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 199810
ED Entered STN: 20 Oct 1998
Last Updated on STN: 3 Mar 2000
Entered Medline: 8 Oct 1998
AB Smooth muscle cells (SMC) secrete a serine protease that cleaves insulin-like growth factor (IGF) binding protein (IGFBP)-4 into fragments that have low affinity for IGF-1. When IGFBP-4 is added to monolayer cultures of cell types that do not secrete this protease, IGF-1 stimulation of DNA synthesis is significantly inhibited. In contrast, if cell types that secrete this protease are used, IGFBP-4 is a much less potent inhibitor. These studies were conducted to determine whether proteolysis of IGFBP-4 accounted for its reduced capacity to inhibit IGF-1-stimulated DNA synthesis. The cleavage site in IGFBP-4 that the SMC protease uses was determined to be lysine120, histidine121. A protease-resistant mutant form of IGFBP-4 was prepared, expressed, purified, and tested for biologic activity using porcine SMC cultures. Addition of the protease-resistant mutant resulted in inhibition of DNA and cell migration responses to IGF-1. The inhibition was concentration dependent and was maximal when 500 ng/ml (20 nM) of the mutant was added with 20 ng/ml (2.8 nM) of IGF-1. When the mutant was added in the absence of IGF-1, it had no activity. The results show that cleavage of IGFBP-4 at lysine120, histidine121 results in inactivation of the ability of IGFBP-4 to bind to IGF-1. Creation of a mutant form of IGFBP-4 that was not cleaved by the protease resulted in inhibition of IGF-1-stimulated actions. The results suggest that IGFBP-4 can act as a potent inhibitor of the anabolic effects of IGF-1 and that the variables that regulate protease activity may indirectly regulate IGF-1 actions.

L11 ANSWER 37 OF 51 MEDLINE on STN DUPLICATE 20
AN 1996256783 MEDLINE
DN PubMed ID: 8676497
TI Characterization of the rubella virus nonstructural protease domain and its cleavage site.
AU Chen J P; Strauss J H; Strauss E G; Frey T K
CS Department of Biology, Georgia State University, Atlanta, Georgia, 30303, USA.
NC AI10793 (United States NIAID NIH HHS)
AI21389 (United States NIAID NIH HHS)
SO Journal of virology, (1996 Jul) Vol. 70, No. 7, pp. 4707-13.
Journal code: 0113724. ISSN: 0022-538X.
Report No.: NLM-PMC190407.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, U.S. GOV'T, P.H.S.)
LA English
FS Priority Journals
EM 199608

ED Entered STN: 22 Aug 1996
Last Updated on STN: 3 Mar 2000
Entered Medline: 15 Aug 1996
AB The region of the rubella virus nonstructural open reading frame that contains the papain-like cysteine protease domain and its cleavage site was expressed with a Sindbis virus vector. Cys-1151 has previously been shown to be required for the activity of the protease (L. D. Marr, C.-Y. Wang, and T. K Frey, Virology 198:586-592, 1994). Here we show that His-1272 is also necessary for protease activity, consistent with the active site of the enzyme being composed of a catalytic dyad consisting of Cys-1151 and His-1272. By means of radiochemical amino acid sequencing, the site in the polyprotein cleaved by the nonstructural protease was found to follow Gly-1300 in the sequence Gly-1299-Gly-1300-Gly-1301. Mutagenesis studies demonstrated that change of Gly-1300 to alanine or valine abrogated cleavage. In contrast, Gly-1299 and Gly-1301 could be changed to alanine with retention of cleavage, but a change to valine abrogated cleavage. Coexpression of a construct that contains a cleavage site mutation (to serve as a protease) together with a construct that contains a protease mutation (to serve as a substrate) failed to reveal trans cleavage. Coexpression of wild-type constructs with protease-mutant constructs also failed to reveal trans cleavage, even after extended in vitro incubation following lysis. These results indicate that the protease functions only in cis, at least under the conditions tested.

L11 ANSWER 38 OF 51 MEDLINE on STN DUPLICATE 21
AN 1996256740 MEDLINE
DN PubMed ID: 8676454
TI Separate functional domains of the herpes simplex virus type 1 protease: evidence for cleavage inside capsids.
AU Robertson B J; McCann P J 3rd; Matusick-Kumar L; Newcomb W W; Brown J C; Colonno R J; Gao M
CS Department of Virology, Bristol-Myers Squibb Pharmaceutical Research Institute, Wallingford, Connecticut 06492-7660, USA.
SO Journal of virology, (1996 Jul) Vol. 70, No. 7, pp. 4317-28.
Journal code: 0113724. ISSN: 0022-538X.
Report No.: NLM-PMC190364.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, U.S. GOV'T, NON-P.H.S.)
LA English
FS Priority Journals
EM 199608
ED Entered STN: 22 Aug 1996
Last Updated on STN: 3 Mar 2000
Entered Medline: 15 Aug 1996
AB The herpes simplex virus type 1 (HSV-1) protease (Pra) and related proteins are involved in the assembly of viral capsids and virion maturation. Pra is a serine protease, and the active-site residue has been mapped to amino acid (aa) 129 (Ser). This 635-aa protease, encoded by the UL26 gene, is autoproteolytically processed at two sites, the release (R) site between amino acid residues 247 and 248 and the maturation (M) site between residues 610 and 611. When the protease cleaves itself at both sites, it releases Nb, the catalytic domain (N0), and the C-terminal 25 aa. ICP35, a substrate of the HSV-1 protease, is the product of the UL26.5 gene. As it is translated from a Met codon within the UL26 gene, ICP35 cd are identical to the C-terminal 329-aa sequence of the protease and are trans cleaved at an identical C-terminal site to generate ICP35 e,f and a 25-aa peptide. Only fully processed Pra (N0 and Nb) and ICP35 (ICP35 e,f) are present in B capsids, which are believed to be precursors of

mature virions. Using an R-site mutant A247S virus, we have recently shown that this mutant protease retains enzymatic activity but fails to support viral growth, suggesting that the release of N0 is required for viral replication. Here we report that another mutant protease, with an amino acid substitution (Ser to Cys) at the active site, can complement the A247S mutant but not a protease deletion mutant. Cell lines expressing the active-site mutant protease were isolated and shown to complement the A247S mutant at the levels of capsid assembly, DNA packaging, and viral growth. Therefore, the complementation between the R-site mutant and the active-site mutant reconstituted wild-type Pra function. One feature of this intragenic complementation is that following sedimentation of infected-cell lysates on sucrose gradients, both N-terminally unprocessed and processed proteases were isolated from the fractions where normal B capsids sediment, suggesting that proteolytic processing occurs inside capsids. Our results demonstrate that the HSV-1 protease has distinct functional domains and some of these functions can complement in trans.

L11 ANSWER 39 OF 51 MEDLINE on STN DUPLICATE 22
AN 1996068694 MEDLINE
DN PubMed ID: 7479877
TI Shared functions in vivo of a glycosyl-phosphatidylinositol-linked aspartyl protease, Mkc7, and the proprotein processing protease Kex2 in yeast.
AU Komano H; Fuller R S
CS Department of Biochemistry, Stanford University School of Medicine, CA 94305-5307, USA.
NC GM39697 (United States NIGMS NIH HHS)
SO Proceedings of the National Academy of Sciences of the United States of America, (1995 Nov 7) Vol. 92, No. 23, pp. 10752-6.
Journal code: 7505876. ISSN: 0027-8424.
Report No.: NLM-PMC40690.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
(RESEARCH SUPPORT, U.S. GOV'T, P.H.S.)
LA English
FS Priority Journals
OS GENBANK-U14733
EM 199512
ED Entered STN: 24 Jan 1996
Last Updated on STN: 3 Mar 2000
Entered Medline: 21 Dec 1995
AB The MKC7 gene was isolated as a multicopy suppressor of the cold-sensitive growth phenotype of a yeast kex2 mutant, which lacks the protease that cleaves pro-alpha-factor and other secretory proproteins at pairs of basic residues in a late Golgi compartment in yeast. MKC7 encodes an aspartyl protease most closely related to product of the YAP3 gene, a previously isolated multicopy suppressor of the pro-alpha-factor processing defect of a kex2 null. Multicopy MKC7 suppressed the alpha-specific mating defect of a kex2 null as well as multicopy YAP3 did, but multicopy YAP3 was a relatively weak suppressor of kex2 cold sensitivity. Overexpression of MKC7 resulted in production of a membrane-associated proteolytic activity that cleaved an internally quenched fluorogenic peptide substrate on the carboxyl side of a Lys-Arg site. Treatment with phosphatidylinositol-specific phospholipase C shifted Mkc7 activity from the detergent to the aqueous phase in a Triton X-114 phase separation, indicating that membrane attachment of Mkc7 is mediated by a glycosyl-phosphatidylinositol anchor. Although disruption of MKC7 or YAP3 alone resulted in no observable

phenotype, mkc7 yap3 double disruptants exhibited impaired growth at 37 degrees C. Disruption of MKC7 and YAP3 in a kex2 null mutant resulted in profound temperature sensitivity and more generalized cold sensitivity. The synergism of mkc7, yap3, and kex2 null mutations argues that Mkc7 and Yap3 are authentic processing enzymes whose functions overlap those of Kex2 in vivo.

L11 ANSWER 40 OF 51 MEDLINE on STN DUPLICATE 23
AN 1995181426 MEDLINE
DN PubMed ID: 7533161
TI Cleavage analysis of insulin-like growth factor (IGF)-dependent IGF-binding protein-4 proteolysis and expression of protease-resistant IGF-binding protein-4 mutants.
AU Conover C A; Durham S K; Zapf J; Masiarz F R; Kiefer M C
CS Endocrine Research Unit, Mayo Clinic, Rochester, Minnesota 55905.
NC DK-07352 (United States NIDDK NIH HHS)
DK-43258 (United States NIDDK NIH HHS)
SO The Journal of biological chemistry, (1995 Mar 3) Vol. 270, No. 9, pp. 4395-400.
Journal code: 2985121R. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
(RESEARCH SUPPORT, U.S. GOV'T, P.H.S.)
LA English
FS Priority Journals
EM 199504
ED Entered STN: 19 Apr 1995
Last Updated on STN: 3 Mar 2000
Entered Medline: 5 Apr 1995
AB Cultured human fibroblasts and osteoblast-like cells secrete an insulin-like growth factor (IGF)-dependent protease that cleaves IGF-binding protein-4 (IGFBP-4) into two fragments of approximately 18 and 14 kDa. Edman degradation of the isolated proteins established the amino termini of the reaction products. Sequence analysis of the 14-kDa carboxyl-terminal half of IGFBP-4 suggested cleavage after methionine at position 135 of the mature protein. Four variant IGFBP-4 molecules with single amino acid substitutions around this cleavage site were constructed and expressed. Wild-type and mutant IG-FBPs-4 bound IGF-I and IGF-II with equivalent affinities and, in the intact state, were equally effective inhibitors of IGF-I action. However, the IGFBP-4 mutants were relatively resistant to IGF-dependent proteolysis. A 5-6-h incubation in human fibroblast conditioned medium in the presence of IGF-II was sufficient for near total hydrolysis of wild-type IGFBP-4, whereas the mutant IGFBPs-4 were only minimally affected at this time. After a 24-h incubation with IGF-II, all mutant IGFBPs-4 showed extensive proteolysis, generating 18- and 14-kDa fragments. Pre-exposure of human fibroblasts in serum-free conditioned medium to IGF-II for 5 h potentiated subsequent IGF-I stimulation of DNA synthesis. When added with IGF-II, the protease-resistant mutant IG-FBPs-4, but not wild-type IGFBP-4, suppressed IGF-II enhancement of IGF-I-stimulated DNA synthesis. These biological studies suggest that the IGFBP-4/IGFBP-4 protease system may play a role modulating local cellular response to IGF-I.

L11 ANSWER 41 OF 51 MEDLINE on STN DUPLICATE 24
AN 1995264461 MEDLINE
DN PubMed ID: 7745718
TI Assembly of the herpes simplex virus capsid: requirement for the carboxyl-terminal twenty-five amino acids of the proteins encoded by the UL26 and UL26.5 genes.

AU Thomsen D R; Newcomb W W; Brown J C; Homa F L
CS Upjohn Company, Kalamazoo, Michigan 49001, USA.
SO Journal of virology, (1995 Jun) Vol. 69, No. 6, pp. 3690-703.
Journal code: 0113724. ISSN: 0022-538X.
Report No.: NLM-PMC189085.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199506
ED Entered STN: 21 Jun 1995
Last Updated on STN: 3 Mar 2000
Entered Medline: 13 Jun 1995
AB Herpes simplex virus type 1 (HSV-1) intermediate capsids are composed of seven proteins, VP5, VP19C, VP21, VP22a, VP23, VP24, and VP26, and the genes that encode these proteins, UL19, UL38, UL26, UL26.5, UL18, UL26, and UL35, respectively. The UL26 gene encodes a protease that cleaves itself and the product of the UL26.5 gene at a site (M site) 25 amino acids from the C terminus of these two proteins. In addition, the protease cleaves itself at a second site (R site) between amino acids 247 and 248. Cleavage of the UL26 protein gives rise to the capsid proteins VP21 and VP24, and cleavage of the UL26.5 protein gives rise to the capsid protein VP22a. Previously we described the production of HSV-1 capsids in insect cells by infecting the cells with recombinant baculoviruses expressing the six capsid genes (D. R. Thomsen, L. L. Roof, and F. L. Homa, J. Virol. 68:2442-2457, 1994). Using this system, we demonstrated that the products of the UL26 and/or UL26.5 genes are required as scaffolds for assembly of HSV-1 capsids. To better understand the functions of the UL26 and UL26.5 proteins in capsid assembly, we constructed baculoviruses that expressed altered UL26 and UL26.5 proteins. The ability of the altered UL26 and UL26.5 proteins to support HSV-1 capsid assembly was then tested in insect cells. Among the specific mutations tested were (i) deletion of the C-terminal 25 amino acids from the proteins coded for by the UL26 and UL26.5 genes; (ii) mutation of His-61 of the UL26 protein, an amino acid required for protease activity; and (iii) mutation of the R cleavage site of the UL26 protein. Analysis of the capsids formed with wild-type and mutant proteins supports the following conclusions: (i) the C-terminal 25 amino acids of the UL26 and UL26.5 proteins are required for capsid assembly; (ii) the protease activity associated with the UL26 protein is not required for assembly of morphologically normal capsids; and (iii) the uncleaved forms of the UL26 and UL26.5 proteins are employed in assembly of 125-nm-diameter capsids; cleavage of these proteins occurs during or subsequent to capsid assembly. Finally, we carried out in vitro experiments in which the major capsid protein VP5 was mixed with wild-type or truncated UL26.5 protein and then precipitated with a VP5-specific monoclonal antibody.(ABSTRACT TRUNCATED AT 400 WORDS)

L11 ANSWER 42 OF 51 MEDLINE on STN DUPLICATE 25
AN 1995264435 MEDLINE
DN PubMed ID: 7745693
TI Foot-and-mouth disease virus Lb proteinase can stimulate rhinovirus and enterovirus IRES-driven translation and cleave several proteins of cellular and viral origin.
AU Ziegler E; Borman A M; Kirchweger R; Skern T; Kean K M
CS Unite de Virologie Moleculaire (CNRS URA 1966), Institut Pasteur, Paris, France.
SO Journal of virology, (1995 Jun) Vol. 69, No. 6, pp. 3465-74.
Journal code: 0113724. ISSN: 0022-538X.
Report No.: NLM-PMC189059.
CY United States

DT Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LA English
FS Priority Journals
EM 199506
ED Entered STN: 21 Jun 1995
Last Updated on STN: 3 Mar 2000
Entered Medline: 13 Jun 1995
AB Rhinovirus and enterovirus 2A proteinases stimulate translation initiation driven from the cognate internal ribosome entry segment (IRES) (S. J. Hambidge and P. Sarnow, Proc. Natl. Acad. Sci. USA 89:10272-10276, 1992; H.-D. Liebig, E. Ziegler, R. Yan, K. Hartmuth, H. Klump, H. Kowalski, D. Blaas, W. Sommergruber, L. Frasel, B. Lamphear, R. Rhoads, E. Kuechler, and T. Skern, Biochemistry 32:7581-7588, 1993). Given the functional similarities between the foot-and-mouth disease virus (FMDV) L proteinase and these 2A proteinases (autocatalytic excision from the nascent viral polyprotein and cleavage of eIF-4 gamma), we investigated whether the FMDV L proteinase would also be able to stimulate translation initiation. We found that purified recombinant FMDV Lb proteinase could stimulate in vitro translation driven from a rhinovirus or enterovirus IRES by 5- to 10-fold. In contrast, stimulation of translation initiation on a cardiovirus IRES by this proteinase was minimal, and stimulation of translation driven from the cognate FMDV IRES could not be evidenced. Studies using an inhibitor or a mutant Lb proteinase indicated that stimulation of IRES-driven translation is mediated via proteolysis of some cellular component(s). Our studies also demonstrated that the Lb proteinase is capable of stimulating initiation of translation on an uncapped cellular message. Unexpectedly, and in contrast to the 2A proteinases, the Lb proteinase specifically cleaved the products of the two reporter genes used in this study: Xenopus laevis cyclin B2 and influenza virus NS. Therefore, we also set out to investigate the requirements for substrate recognition by the Lb proteinase. Purified recombinant Lb proteinase recognized at least one mengovirus polypeptide and specifically cleaved human cyclin A and poliovirus replicase-related polypeptides. In the latter case, the site(s) of cleavage was located within the N-terminal part of polypeptide 3D. Sequence comparisons revealed no significant primary sequence similarities between the target proteins and the two sites already known to be recognized by the FMDV L proteinase.

L11 ANSWER 43 OF 51 MEDLINE on STN DUPLICATE 26
AN 1994197861 MEDLINE
DN PubMed ID: 8148011
TI Proteolysis in heterocyst-forming cyanobacteria: characterization of a further enzyme with trypsin-like specificity, and of a prolyl endopeptidase from Anabaena variabilis.
AU Strohmeier U; Gerdes C; Lockau W
CS Institut fur Botanik der Universitat, Regensburg, Bundesrepublik Deutschland.
SO Zeitschrift fur Naturforschung. C, Journal of biosciences, (1994 Jan-Feb) Vol. 49, No. 1-2, pp. 70-8.
Journal code: 8912155. ISSN: 0939-5075.
CY GERMANY: Germany, Federal Republic of
DT Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LA English
FS Priority Journals
EM 199405
ED Entered STN: 23 May 1994
Last Updated on STN: 3 Mar 2000
Entered Medline: 11 May 1994

AB Soluble extracts of the cyanobacterium Anabaena variabilis ATCC 29413 and an engineered mutant that lacks an intracellular protease cleaving after Lys and Arg (Maldener, Lockau, Cai, and Wolk, Mol. Gen, Genet. 225, 113-120 (1991)) were separated by ion exchange chromatography, and protease profiles determined using azocasein, N alpha-benzoyl-D,L-arginine-4-nitroanilide and N-carbobenzoxy-glycyl-L-proline-4-nitroanilide as substrates. A second enzyme cleaving at the carboxyl side of lysine and arginine, and a prolyl endopeptidase were detected, enriched and characterized. Both proteolytic enzymes appear to be located in the periplasm.

L11 ANSWER 44 OF 51 MEDLINE on STN DUPLICATE 27
AN 1994285789 MEDLINE
DN PubMed ID: 7516997
TI A conserved Streptococcus pyogenes extracellular cysteine protease cleaves human fibronectin and degrades vitronectin.
AU Kapur V; Topouzis S; Majesky M W; Li L L; Hamrick M R; Hamill R J; Patti J M; Musser J M
CS Department of Pathology, Baylor College of Medicine, Houston, Texas 77030.
NC HL-47655 (United States NHLBI NIH HHS)
SO Microbial pathogenesis, (1993 Nov) Vol. 15, No. 5, pp. 327-46.
Journal code: 8606191. ISSN: 0882-4010.
CY ENGLAND: United Kingdom
DT (COMPARATIVE STUDY)
Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
(RESEARCH SUPPORT, U.S. GOV'T, P.H.S.)
LA English
FS Priority Journals
OS GENBANK-L26125; GENBANK-L26126; GENBANK-L26127; GENBANK-L26128; GENBANK-L26129; GENBANK-L26130; GENBANK-L26131; GENBANK-L26132; GENBANK-L26133; GENBANK-L26134; GENBANK-L26135; GENBANK-L26136; GENBANK-L26137; GENBANK-L26138; GENBANK-L26139; GENBANK-L26140; GENBANK-L26141; GENBANK-L26142; GENBANK-L26143; GENBANK-L26144; GENBANK-L26145; GENBANK-L26146; GENBANK-L26147; GENBANK-L26148; GENBANK-L26149; GENBANK-L26150; GENBANK-L26151; GENBANK-L26152; GENBANK-L26153; GENBANK-L26154; +
EM 199407
ED Entered STN: 10 Aug 1994
Last Updated on STN: 3 Mar 2000
Entered Medline: 28 Jul 1994
AB Streptococcus pyogenes secretes an extracellular cysteine protease that cleaves human interleukin 1 beta precursor to form biologically active IL-1 beta, a major cytokine mediating inflammation and shock. To further investigate the potential role of the cysteine protease in host-parasite interactions, the enzyme was purified to apparent homogeneity and tested for ability to degrade several human extracellular matrix proteins. Purified protease cleaved fibronectin, apparently at specific sites, and rapidly degraded vitronectin. In contrast, the protease did not have substantial activity against laminin. The cysteine protease also cleaved fibronectin from human umbilical vein endothelial cells grown in vitro. Allelic variation in the cysteine protease structural gene was studied in 67 strains expressing 39 M protein serotypes and five provisional M serologic types, and representing 50 phylogenetically distinct clones identified by multilocus enzyme electrophoresis. The gene is well conserved and allelic variation is due solely to accumulation of point mutations. Based on predicted amino acid sequences, one mature cysteine protease variant would be made by clones expressing serotypes M2, M3, M4, M5, M6, M9, M10, M11, M12, M14, M18, M22, M23, M25, M27, M41, M49, M56, M59, two provisional M types, and two clones

non-typeable for M protein. Moreover, 33 of the 39 speB alleles identified encode one of three mature protease variants that differ from one another at only one or two amino acids clustered in a ten-amino acid region. All 39 alleles, and virtually all strains, encode a product that reacts with polyclonal antisera specific for purified cysteine protease. No compelling evidence was found for a primitive differentiation of the speB gene into two distinct classes, as has been proposed for M protein, opacity factor phenotype, and vir regulon architecture. The results demonstrate that the cysteine protease is well conserved in natural populations of S. pyogenes, provide additional evidence that this enzyme is involved in host-parasite interactions, and suggest that the protease plays a role in bacterial dissemination, colonization, and invasion, and inhibition of wound healing.

L11 ANSWER 45 OF 51 MEDLINE on STN DUPLICATE 28
AN 1992292244 MEDLINE
DN PubMed ID: 1602542
TI Effect of retroviral proteinase inhibitors on Mason-Pfizer monkey virus maturation and transmembrane glycoprotein cleavage.
AU Sommerfelt M A; Petteway S R Jr; Dreyer G B; Hunter E
CS Department of Microbiology, University of Alabama, Birmingham 35294.
NC CA 27834 (United States NCI NIH HHS)
SO Journal of virology, (1992 Jul) Vol. 66, No. 7, pp. 4220-7.
Journal code: 0113724. ISSN: 0022-538X.
Report No.: NLM-PMC241225.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, U.S. GOV'T, P.H.S.)
LA English
FS Priority Journals; AIDS
EM 199207
ED Entered STN: 24 Jul 1992
Last Updated on STN: 3 Mar 2000
Entered Medline: 10 Jul 1992
AB Mason-Pfizer monkey virus (M-PMV) is the prototype type D retrovirus which preassembles immature intracytoplasmic type A particles within the infected cell cytoplasm. Intracytoplasmic type A particles are composed of uncleaved polyprotein precursors which upon release are cleaved by the viral proteinase to their constituent mature proteins. This results in a morphological change in the virion described as maturation. We have investigated the role of the viral proteinase in virus maturation and infectivity by inhibiting the function of the enzyme through mutagenesis of the proteinase gene and by using peptide inhibitors originally designed to block human immunodeficiency virus type 1 proteinase activity. Mutation of the active-site aspartic acid, Asp-26, to asparagine abrogated the activity of the M-PMV proteinase but did not affect the assembly of noninfectious, immature virus particles. In mutant virions, the transmembrane glycoprotein (TM) of M-PMV, initially synthesized as a cell-associated gp22, is not cleaved to gp20, as is observed with wild-type virions. This demonstrates that the viral proteinase is responsible for this cleavage event. Hydroxyethylene isostere human immunodeficiency virus type 1 proteinase inhibitors were shown to block M-PMV proteinase cleavage of the TM glycoprotein and Gag-containing precursors in a dose-dependent manner. The TM cleavage event was more sensitive than cleavage of the Gag precursors to inhibition. The infectivity of treated particles was reduced significantly, but experiments showed that inhibition of precursor and TM cleavage may be at least partially reversible. These results demonstrate that the M-PMV aspartyl proteinase is activated in released virions and that the hydroxyethylene isostere proteinase inhibitors used in this study exhibit a broad spectrum of antiretroviral activity.

L11 ANSWER 46 OF 51 MEDLINE on STN DUPLICATE 29
AN 1992237326 MEDLINE
DN PubMed ID: 1570342
TI A genetic system for studying the activity of a proteolytic enzyme.
AU Dasmahapatra B; DiDomenico B; Dwyer S; Ma J; Sadowski I; Schwartz J
CS Antiviral Chemotherapy, Schering-Plough Research Institute, Bloomfield, NJ 07003.
SO Proceedings of the National Academy of Sciences of the United States of America, (1992 May 1) Vol. 89, No. 9, pp. 4159-62.
Journal code: 7505876. ISSN: 0027-8424.
Report No.: NLM-PMC525652.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199205
ED Entered STN: 12 Jun 1992
Last Updated on STN: 29 Jan 1996
Entered Medline: 28 May 1992
AB We describe a genetic system for monitoring the activity of a specific proteolytic enzyme by taking advantage of the properties of the yeast transcriptional activator GAL4. The GAL4 protein contains two separable and functionally essential domains: the amino-terminal DNA binding domain and the carboxyl-terminal transcriptional activating domain. We constructed two hybrid proteins by inserting between the DNA binding domain and the activation domain of GAL4 either (i) a self-cleaving protease (3C protease of a picornavirus, coxsackievirus B3) or (ii) a mutant form of the protease that is unable to cleave. We show that, although the hybrid protein containing the mutant protease activates transcription of GAL1-lacZ reporter gene, the hybrid protein bearing the wild-type protease is proteolytically cleaved and fails to activate transcription. Our approach to monitor the proteolytic activity could be used to develop simple genetic systems to study other proteases.

L11 ANSWER 47 OF 51 MEDLINE on STN DUPLICATE 30
AN 1988251412 MEDLINE
DN PubMed ID: 3289538
TI Mutant isolation and cloning of the gene encoding protease VII from Escherichia coli.
AU Sugimura K
CS Suntory Bio Pharma Tech Center, Gunma, Japan.
SO Biochemical and biophysical research communications, (1988 Jun 16) Vol. 153, No. 2, pp. 753-9.
Journal code: 0372516. ISSN: 0006-291X.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 198807
ED Entered STN: 8 Mar 1990
Last Updated on STN: 8 Mar 1990
Entered Medline: 22 Jul 1988
AB A mutant of Escherichia coli lacking protease VII, the outer membrane-associated protease which specifically cleaves paired basic residues (1), was isolated by using N-methyl-N'-nitro-N-nitrosoguanidine treatment. The mutant exhibited no significant change as for its growth rate and microscopic feature compared with wild cells. The gene encoding protease VII was cloned by using

complementation analysis of protease VII (-) mutation. The minicell experiment showed that the gene encoded a putative precursor protein of 38,000 Mr which was processed into a protein of 36,000 Mr suggesting the presence of a signal peptide on the putative precursor.

L11 ANSWER 48 OF 51 CAPLUS COPYRIGHT 2009 ACS on STN
AN 1987:82679 CAPLUS
DN 106:82679
OREF 106:13549a,13552a
TI Cleavage site mutant as a potential vaccine
AU Homma, Morio
CS Sch. Med., Kobe Univ., Kobe, 650, Japan
SO Concepts Viral Pathog. (1986), Volume 2, 388-93. Editor(s): Notkins, Abner Louis; Oldstone, Michael B. A. Publisher: Springer, New York, N. Y. CODEN: 52MXA4
DT Conference; General Review
LA English
AB A review with 21 refs. Paramyxoviruses and influenza viruses become activated and replicate in multiple cycles when the envelope glycoprotein of the virus is cleaved by a host protease. In the absence of protease, the replication is limited to a single cycle. A protease activation mutant of Sendai virus was obtained, whose replication is restricted to a single cycle in the lung of mice, but which nevertheless, induces immunity. The availability of such mutants for vaccines, their strengths and limitations are discussed.

L11 ANSWER 49 OF 51 MEDLINE on STN DUPLICATE 31
AN 1983290793 MEDLINE
DN PubMed ID: 6350278
TI Temperature-sensitive prolipoprotein signal peptidase in an Escherichia coli mutant: use of the mutant for an efficient and convenient assay system.
AU Yamagata H
SO Journal of biochemistry, (1983 Jun) Vol. 93, No. 6, pp. 1509-15. Journal code: 0376600. ISSN: 0021-924X.
CY Japan
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 198310
ED Entered STN: 19 Mar 1990
Last Updated on STN: 3 Mar 2000
Entered Medline: 8 Oct 1983
AB Escherichia coli mutant Y815 accumulates the precursor of lipoprotein (prolipoprotein) in its envelope. The accumulated prolipoprotein could be chased to mature lipoprotein at 30 degrees C but not at 60 degrees C (Yamagata, H., Ippolito, C., Inukai, M., & Inouye, M. (1982) J. Bacteriol. 152, 1163). When the envelope fraction prepared from the mutant was mixed with the envelope fraction prepared from wild-type E. coli cells and incubated at 60 degrees C in the presence of Triton X-100, the prolipoprotein in the mutant envelope fraction was cleaved rapidly to mature lipoprotein. The cleavage was dependent on the addition of wild-type envelope fraction and Triton X-100 to the reaction mixture. This indicated that the prolipoprotein accumulated in the mutant envelope is a good substrate for the signal peptidase which cleaves the signal peptide from the prolipoprotein, and hence the accumulation of prolipoprotein was due to lack of the signal peptidase in the mutant. The optimum concentration of Triton X-100 for the cleavage of the prolipoprotein in the above in vitro system was 0.05 to 0.1% (v/v) at a wild-type envelope concentration of 0.35 mg protein/ml. Prolipoprotein accumulated in wild-type cells on

treatment with globomycin, a specific inhibitor of the signal peptidase, was also cleaved to mature lipoprotein under the same conditions. Triton X-100 was shown to solubilize the signal peptidase from the envelope fraction. The cleavage of the prolipoprotein was rapid and complete in the in vitro system described here, which provides an efficient and convenient assay system for the solubilized signal peptidase for prolipoprotein.

L11 ANSWER 50 OF 51 MEDLINE on STN DUPLICATE 32
AN 1976005412 MEDLINE
DN PubMed ID: 51020
TI Proteinase C (carboxypeptidase Y) mutant of yeast.
AU Wolf D H; Fink G R
SO Journal of bacteriology, (1975 Sep) Vol. 123, No. 3, pp. 1150-6.
Journal code: 2985120R. ISSN: 0021-9193.
Report No.: NLM-PMC235840.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 197512
ED Entered STN: 13 Mar 1990
Last Updated on STN: 13 Mar 1990
Entered Medline: 4 Dec 1975
AB A mutant of yeast lacking proteinase C (carboxypeptidase Y) activity has been found by using a histochemical stain to screen mutagenized colonies. This defect segregates 2:2 in meiotic tetrads. Cell extracts lacked the esterolytic, amidase, and proteolytic activities associated with proteinase C. The absence of proteinase C does not affect mitotic growth and has no obvious effect on the formation of viable ascospores or meiotic segregation. The mutant grows on peptides known to be cleaved by proteinase C in vitro. This finding is consistent with the idea that other enzymes exist in vivo with overlapping substrate specificities.

L11 ANSWER 51 OF 51 CAPLUS COPYRIGHT 2009 ACS on STN
AN 1971:39504 CAPLUS
DN 74:39504
OREF 74:6341a,6344a
TI Peptidases in Escherichia coli K-12 capable of cleaving lysine homopeptides
AU Sussman, Arthur J.; Gilvarg, Charles
CS Dep. Biochem. Sci., Princeton Univ., Princeton, NJ, USA
SO Journal of Biological Chemistry (1970), 245(24), 6518-24
CODEN: JBCHA3; ISSN: 0021-9258
DT Journal
LA English
AB The peptidases present in E. coli K-12 that can cleave lysine oligopeptides were characterized to establish a system in which one could examine questions relating to peptidase specificity, function, and regulation. As an aid to this study, a mutant with diminished peptidase activity was isolated with the use of the reduced ability to cleave trilysine as a screening procedure. In this simplified system, the remaining enzymic capacity to cleave lysine homopeptides was then characterized. On the basis of substrate specificities and cofactor requirements as well as reolution of the enzymes by ion exchange and Sephadex chromatog., it ws shown that the mutant contains the following lysine peptidases: (a) a Co2+ dependent peptidase capable of splitting dilysine, (b) an EDTA sensitive peptidase specific for trilysine, and (c) an endopeptidase that can cleave tetralysine but has no activity toward di- or trilysine. The parental K-12 strain contains, in addition to these 3

activities, a metal independent peptidase capable of
cleaving all 3 lysine peptides.
OSC.G 1 THERE ARE 1 CAPLUS RECORDS THAT CITE THIS RECORD (1 CITINGS)

=> S (protease or proteinase or peptidase) (4A) (substrate binding)
L12 483 (PROTEASE OR PROTEINASE OR PEPTIDASE) (4A) (SUBSTRATE BINDING)

=> s l11 and l12
L13 1 L11 AND L12

=> d l13 bib ab

L13 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2009 ACS on STN
AN 1998:485878 CAPLUS
DN 129:227431
OREF 129:46169a,46172a
TI Engineering the S1' subsite of trypsin: Design of a protease which cleaves between dibasic residues
AU Kurth, Torsten; Grahn, Sibylla; Thormann, Michael; Ullmann, Dirk; Hofmann, Hans-Joerg; Jakubke, Hans-Dieter; Hedstrom, Lizbeth
CS Department of Biochemistry, Brandeis University, Waltham, MA, 02454, USA
SO Biochemistry (1998), 37(33), 11434-11440
CODEN: BICHAW; ISSN: 0006-2960
PB American Chemical Society
DT Journal
LA English
AB Here, trypsin was redesigned by protein engineering into a site-specific protease which hydrolyzes peptides between dibasic residues. Trypsin normally exhibits high S1 specificity for Arg and Lys residues. However, the S1' specificity of trypsin is very broad, with only a slight preference for hydrophobic residues in P1'. Here, the authors replaced Lys-60 with Glu and Asp to introduce a high specificity for basic residues into the S1' site of trypsin. Both mutations caused a dramatic increase in S1' specificity for Arg and Lys as measured by acyl transfer reactions. In mutant K60E, the preference for Arg increased 70-fold, whereas the preference for P1'-Lys increased 12-fold. In contrast, the preferences for other P1' residues either decreased slightly or remained the same. Thus, K60E prefers P1'-Arg over most other P1' residues by 2 orders of magnitude. Similar results were obtained when P1' specificity was measured in peptide cleavage assays. K60D exhibited an S1' specificity profile very similar to that of K60E, although the P1'-Arg preference was reduced by a factor of 2.5. Mol. modeling studies suggested that the high S1' specificity for Arg in K60E may be due to the formation of a salt bridge between Glu-60 and P1'-Arg of the substrate.
OSC.G 22 THERE ARE 22 CAPLUS RECORDS THAT CITE THIS RECORD (22 CITINGS)
RE.CNT 25 THERE ARE 25 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

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